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THE EFFECTS OF CIGARETTE SMOKING ON METABOLIC RATE, HEART RATE, OXYGEN PULSE, AND BREATHING RATE

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OUR investigation was originally undertaken as a study of the immediate effects of smoking on metabolic rate and heart rate. Thirty-nine persons were used, 18 men and 21 women. They were grouped as (1) heavy smokers, (2) light smokers, and (3) abstainers. In this series no attempt was made to insure basal metabolic conditions, although no determinations were made directly after meals.

A second series of observations was carried out to determine the effects over a longer period of time. Twenty persons were studied, all in a basal metabolic state. After a preliminary determination of basal metabolic rate, heart rate, breathing rate, and oxygen pulse, the person was asked to smoke a cigarette, expending no more effort than was necessary. A second determination of metabolic rate was made immediately following this, and three more determinations at fifteen-minute intervals thereafter. In all experiments, disturbances, such as bright light, noises, temperature fluctuations, etc., were avoided. Subjective changes reported by the person were noted, as well as the manner of smoking, i.e., whether or not smoke was inhaled.

The apparatus used was a late model McKesson Metabolor. Face masks were used rather than the rubber bit and nose clamp in order to afford the person greater comfort and to facilitate normal breathing.

RESULTS

The data obtained in these experiments are shown in the accompanying tables and figures. The persons used in determining immediate effects of smoking (Table I, *A*) represent an average group of individuals, including heavy

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smokers, light smokers, and abstainers. Determinations of metabolic rate were not made under basal (postabsorptive) conditions. The 20 persons used in determining long time effects of smoking were all habitual smokers. Each was in a basal condition and none had smoked since the previous day.

TABLE I

EFFECT OF SMOKING ONE CIGARETTE

A, Immediate effects. *B*, Effects over a 45-minute period. Metabolic rate (calories per square meter per hour); heart rate (beats per minute); oxygen pulse (c.c. oxygen per minute per heart beats per minute); breathing rate (inhalations per minute).

	METABOLIC RATE	HEART RATE	OXYGEN PULSE	BREATHING RATE
A: 39 Persons (Not Basal) Immediate Effects of Smoking				
Previous to smoking	35.1	67.6	3.8	12.2
Immediately after smoking	38.3	71.8	3.7	12.1
Percentage change	8.8	6.2	-2.9	-0.7
B: 20 Persons (All Basal) All Habitual Smokers				
Previous to smoking	31.8	61.6	3.4	12.2
Immediately after smoking	36.3	71.9	3.3	11.3
Percentage change	14.3	16.7	-2.1	-7.4
15 minutes after smoking	34.7	62.8	3.5	11.6
Percentage change	9.3	1.9	5.7	-4.4
30 minutes after smoking	34.7	60.4	3.6	11.6
Percentage change	9.2	-1.9	8.4	-4.6
45 minutes after smoking	35.7	60.0	3.7	12.0
Percentage change	12.4	-2.6	11.0	-1.1

Table I and Fig. 1 show only the average effects and give no indication of the extremes. The greatest increase in metabolic rate in this series occurred in a woman who is a confirmed smoker and inhales the smoke deeply. Her metabolic rate rose 40.7 per cent immediately after smoking. It may be added that in one individual in a state of nervous tension and emotional instability, a rise of 84 per cent was observed; this person was excluded from the data as abnormal. Five individuals showed a decrease in metabolic rate. One individual in the second series (basal persons) showed a decrease, the rate of metabolism falling 8.5 per cent immediately after smoking, continuing to fall fifteen minutes later, after which it returned to nearly normal.

TABLE II

IMMEDIATE EFFECTS OF SMOKING IN 39 PERSONS

Percentage of total cases showing increase, decrease, and no change in metabolic rate, heart rate, oxygen pulse, and breathing rate.

	METABOLIC RATE	HEART RATE	OXYGEN PULSE	BREATHING RATE
Percentage of persons showing increase	82.0	71.8	55.3*	41.0
Percentage of persons showing decrease	12.8	25.6	44.7	53.9
Percentage of persons showing no change	5.1	2.5	0.0	5.1

*A greater percentage shows an increase in oxygen pulse, yet Fig. 1 shows a decrease after smoking. This is due to the more profound drop in some persons rather than the rise in others.

By the distribution graph (Fig. 2) it can be seen that in the greatest number of cases an increase in metabolic rate of from 5 to 10 per cent occurred. The average increase was 8.9 per cent. In three cases the maximal metabolic rate

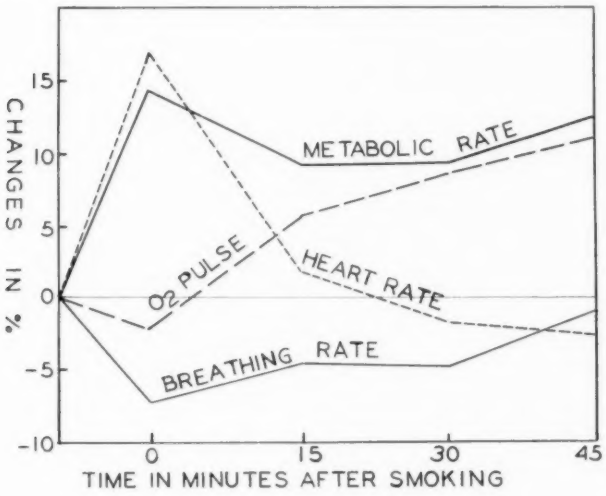

Fig. 1.—Per cent changes in metabolic rate, heart rate, oxygen pulse, and breathing rate over a forty-five-minute period after smoking. Average for 20 persons in basal metabolic state.

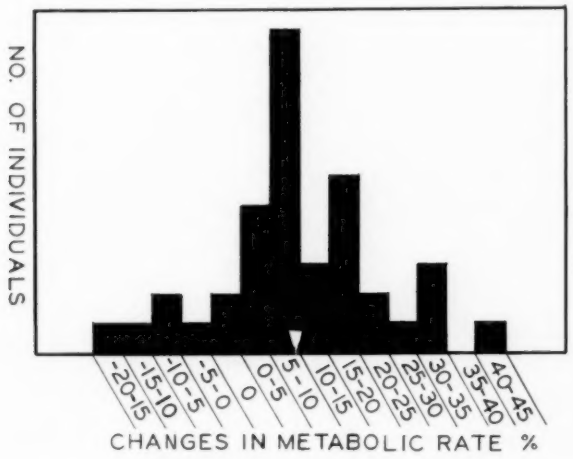

Fig. 2.—Distribution of changes in metabolic rate immediately following smoking in 39 persons. Arrow indicates the average.

was reached fifteen minutes after smoking, in three cases at thirty minutes, and in four cases at forty-five minutes. The remaining ten cases showed the maximal metabolism immediately after smoking. Most of the persons showed a second slight rise at about forty-five minutes. In Table II are indicated the percentages of total cases that showed an increase, a decrease, or no change in metabolic rate, heart rate, oxygen pulse, and breathing rate, immediately following smoking. Typically there occurred simultaneously an increase in metabolic rate, an increase in heart rate, a decrease in breathing rate, and a temporary decrease in oxygen pulse, followed by a rise back to and above the normal level.

A comparison of effects of smoking on men and women was made. The average increase in metabolic rate for 18 men was 7.7 per cent; the average

increase for 21 women was 9.9 per cent. The average increase in heart rate for men was 5.9 per cent, while that for women was 6.4 per cent.

In general, there was a tendency for those who inhaled the most smoke to show the greatest physiologic changes. Profound changes also occurred among abstainers and among those who smoked only occasionally, doubtless due to the lack of acquired tolerance. Habitual smokers who inhaled little or no smoke tend to show only moderate effects.

DISCUSSION

It is obvious that many factors contribute to the physiologic changes due to smoking, including the individual variation in method of smoking and tolerance for nicotine, the state of health and emotional stability of the subject, the psychic factors involved in making the determinations, the form of tobacco used, and the presence of substances other than nicotine in tobacco.

In this series of experiments psychic factors were reduced to a minimum, though such factors might well be considered important in individuals smoking for the first time, or in those individuals who rarely smoke. In the second group of experiments in which tests were made over a forty-five-minute period, all persons were accustomed to cigarette smoking. No information was given the subjects as to the type of effect to be expected. It will be noted from Table I that persons in a basal state showed more profound changes than did the others. This is in accord with the observations of most smokers that smoking produces greater subjective changes before meals than after meals. It has been found also (Haggard and Greenberg, 1934) that changes in blood sugar and respiratory quotient are greatest in a fasting condition.

The oxygen pulse almost invariably undergoes a diminution immediately after smoking, followed by a rise to a level considerably higher than normal, indicating an increase in oxygen used per stroke of the heart. The initial fall may be attributed to the great increase in heart rate and the resulting decrease in stroke volume occurring before the force of the beat had improved proportionally.

In persons in a basal condition, the effects of smoking one cigarette persist for at least forty-five minutes. The trend of the curves (Fig. 1) indicates that these effects must continue over a still longer period; how long is not shown by the present experiments.

Effects of cigarette smoking are due almost solely to absorbed nicotine, although other volatile substances, such as pyridine and collidine, are present in tobacco smoke, together with some hydrocyanic acid and carbon monoxide (Sollmann). Bodnár, Nagy, and Dickmann (1935) report that 6 per cent of the nicotine is absorbed by "noninhalers," 1.7 per cent of which is excreted in the urine, and that 93 per cent of the nicotine is absorbed by "inhalers," none of which is excreted in the urine. Thus a tolerance is developed which might well account for the great effects sometimes apparent in nonhabituated smokers. It has been demonstrated by many that nicotine in sufficiently large doses stimulates adrenalin secretion and hyperglycemia. Dill, Edwards, and Forbes (1934) found that in 90 per cent of the 60 observations on smoking in ten subjects, the blood sugar level varied within only 5 per cent of the resting

level without significant trend up or down. They found also that blood lactic acid was not varied by smoking. Oxygen consumption was found to increase, in some subjects from 5 to 10 per cent, this being in accord with our results. McCormick (1935) believes the rise in blood sugar is a direct response of the adrenals to the presence of a toxin. He offers the evidence that resistance to nicotine poisoning is greatly increased by previous administration of adrenalin.

Recently Short and Johnson (1939) have shown in a study of five subjects that the changes in pulse rate, blood pressure, and skin temperature due to smoking cigarettes continuously could be duplicated by subcutaneous injection of adrenalin solution (1 c.c. of 1:1,000). They concluded that nicotine effects can be explained by an increased output of adrenalin. Whether the effects of smoking are due only, if at all, to liberation of adrenalin or to other pharmacologic reactions of nicotine will have to be settled when our knowledge is more complete.

CONCLUSIONS

1. Cigarette smoking caused an increase in metabolic rate in 82 per cent of 39 subjects. In 13 per cent a decrease occurred. In 5 per cent no immediate effects were observed. The average effect on metabolic rate was an increase of 8.9 per cent.

2. The maximum effect on basal metabolism of smoking one cigarette was reached immediately in some cases, and was delayed as long as forty-five minutes in others.

3. The first rise in metabolic rate was typically followed by a second increase, reaching its summit about forty-five minutes later.

4. Smoking caused an increase in heart rate in 72 per cent of the persons, a decrease in 26 per cent, and no change in 2.5 per cent. After fifteen minutes the heart rate became slower than normal.

5. The rate of breathing decreased immediately after smoking, returning to normal in about forty-five minutes.

6. Smoking caused an immediate reduction in the oxygen pulse value, followed by an increase for at least forty-five minutes.

7. Greatest physiologic effects of smoking were shown by confirmed smokers who inhaled the smoke, and by persons who were unaccustomed to smoking.

8. In this study, women showed more marked changes than men.

9. More marked changes occurred in persons in a basal metabolic condition than in persons in a nonbasal metabolic condition.

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A COMPARISON OF THE EFFECTS OF LARGE DOSES OF CALCIUM GLUCONATE-IDONATE, CALCIUM GLUCONATE, AND CALCIUM CHLORIDE*

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THE wide use of intravenous calcium therapy makes the pharmacologic action of a readily soluble calcium salt a matter of some interest. Calcium gluconate-idonate† is a very soluble salt. Hence a study of its action, comparing it with calcium chloride and with calcium gluconate, has been made.

PROCEDURE AND RESULTS

Chronic Studies in Rats.—The drugs in solution or suspension were administered by stomach tube to rats six days weekly throughout the experiment. The animals were weighed at least once a week. Certain tissues from animals dying during the experiment, as well as those surviving for its duration, were examined histologically. Calcium gluconate-idonate, calcium chloride, and calcium gluconate were all given in amounts equivalent to 0.4 Gm. calcium per kilogram of body weight daily, the first two in solution and the third in a heavy suspension. The gluconate-idonate and the chloride were also tested at levels equivalent to 1 and 2 Gm. calcium per kilogram per day. Ten animals comprised each group except that receiving 1 Gm. calcium per kilogram as gluconate-idonate; it contained 20 rats.

All the animals receiving calcium at the 2 Gm. per kilogram level died after a single dose; hence they are not included in Fig. 1, where the average weight of each group is plotted against the survival time in days. A cross bar indicates the death of an animal. Administration of 1 Gm. of calcium per kilogram of body weight as calcium chloride produced death after one or two doses, but as calcium gluconate-idonate 4 animals survived the entire period. In a dose of 0.4 Gm. calcium per kilogram, of the rats receiving calcium chloride 5 of the original 10 survived the entire period, while all those receiving calcium gluconate-idonate or calcium gluconate of the same calcium content survived. Two of the gluconate animals died (at 56 and 63 days, respectively), but the entire gluconate-idonate group was still alive.

Pathology.—The heart, kidney, and liver tissue from animals given calcium gluconate-idonate equivalent to 1 Gm. of calcium per kilogram per

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The calcium gluconate-idonate used in this study was supplied by Charles Pfizer & Co., Inc., who have contributed generously to the support of this research.

†Calcium gluconate-idonate is soluble in water to the extent of 411 Gm. per liter of solution at 25° C. It is possible to prepare a temporarily supersaturated solution of about 45 per cent at room temperature. The calcium content of the salt is approximately 9.3 per cent. Idonic acid is the sugar acid derived from the hexose idose and is isomeric with gluconic acid.

day for seventy days were examined histologically. These tissues were essentially normal in appearance, showing no abnormalities which could be ascribed to the effect of the drug. Likewise, neither calcium gluconate for seventy days nor calcium chloride for sixty-five days each, given in amounts containing 0.4 Gm. of calcium per kilogram per day, produced any apparent change in these tissues.

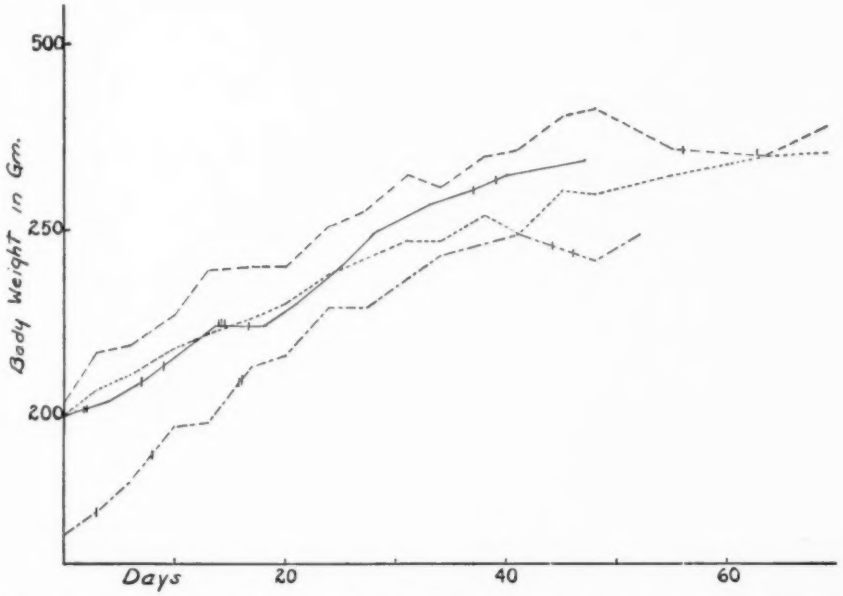

Fig. 1.—Average growth curves of rats receiving indicated doses of calcium salts daily. Cross bars indicate deaths of animals.

— — — — — Calcium chloride = 0.4 Gm. Ca per kg.
- - - - - - - Calcium gluconate = 0.4 Gm. Ca per kg.
---------- Calcium gluconate-idonate = 0.4 Gm. Ca per kg.
———————— Calcium gluconate-idonate = 1.0 Gm. Ca per kg.

Acute Studies.—Eighteen adult dogs were used. The animals received morphine sulfate subcutaneously, 10 mg. per kg. body weight, thirty minutes before injection of the calcium salt was begun. The calcium solution* (0.205 M) was injected through a cannula into the femoral vein at varying rates. A blood sample was withdrawn from the femoral vein just prior to cannulation; a second was obtained at death by direct cardiac puncture. The concentration of calcium in the serum was determined by the method of Kramer and Tisdall.[3] Electrocardiograms† from Lead II were taken at frequent intervals during the injection.

The protocols from all experiments are summarized in Table I. The results of Hoff, Smith, and Winkler,[1, 2] who used the same procedure with calcium chloride, are included for comparison.

*To prepare the calcium gluconate solution it was necessary to heat the mixture on the steam bath to bring about solution. Dextrose 5.0 per cent, citric acid 0.37 per cent, and lactic acid 1.0 per cent were then added to stabilize the supersaturated solution which was filtered and diluted to volume after recooling to room temperature.

Calcium gluconate-idonate in 20 per cent solution (0.507 M) was also injected intravenously into each of 2 dogs in quantities of 3 c.c. per kg. at rates of 1.76 and 2.14 c.c. per kg. per minute without subsequent ill effect.

†Dr. H. E. Hoff and Dr. P. K. Smith kindly assisted in obtaining the electrocardiograms.

TABLE I

	CALCIUM CHLORIDE					CALCIUM GLUCONATE-IDONATE					CALCIUM GLUCONATE				
DOG	KG.	C.C.	C.C./KG.	C.C./KG. MIN.	MG. % CA	KG.	C.C.	C.C./KG.	C.C./KG. MIN.	MG. % CA	KG.	C.C.	C.C./KG.	C.C./KG. MIN.	MG. % CA
1	12.2	99	8.1	0.5	51.4	12.5	193	15.4	0.81	111	12.5	856	68.7	1.01	192[A]
2	9.1	280	30.8	0.7	45.6	9.0	92	10.2	1.12	100	10.8	63	5.8	3.89	182
3	10.5	66	6.3	0.8	53.0	5.8	166	28.6	1.22	231	10.7	110	10.3	1.72	100
4	6.8	149	21.9	0.8	106.4	6.8	296	43.5	1.34	290	10.2	89	8.7	1.29	88
5	12.6	89	7.1	1.0	56.8	6.2	37	6.0	5.97	170	9.0	368	40.9	1.14	175
6	7.5	113	15.1	1.1	70.8	6.9	84	12.2	0.94	76	18.1	368	20.3	0.96	89
7	7.5	142	18.9	1.1	60.0	7.0	214	30.6	1.22	127	12.5	108	8.6	0.86	98
8	8.8	209	23.8	2.6	52.4	10.7	610	57.0	1.66	242[A]	10.1	203	20.1	1.25	152
9	11.6	86	7.4	7.4	95.4	11.6	524	45.2	1.10	168[A]					
10	7.0	155	22.1	1.1	126.0	8.0	120	15.0	5.45	110					
11	17.5	230	13.1	1.1	138.4										
12	6.4	142	22.2	1.6	114.0										
13	9.6	43	4.5	2.6	86.0										
	Mean		15.5		81.2			26.4		162.5			17.8		134.5
	S.D.		8.4		31.8			17.4		71.0			12.32		45.0
	PEm		1.57		5.9			3.7		16.0			3.14		10.7

[A]—died in cardiac arrest.

Changes in the electrocardiogram followed the sequence reported by the above-mentioned authors for calcium chloride. An initial phase of inhibition, both of rate and of auriculoventricular conduction, was followed by a period of excitation, during which ectopic rhythms appeared, and which often culminated in ventricular fibrillation. In experiments in which ventricular fibrillation did not appear during the rapid phase, a second period of inhibition occurred, and the animal died of cardiac arrest. In experiments in which calcium gluconate-idonate or gluconate were employed, certain variations were detected in their effect on the heart. First, corresponding with the greater amounts of calcium required to kill, the duration of the various phases was increased. Second, the degree of inhibition during the first stage was increased, so that arrest of as much as eight to ten seconds was observed. Thus, the tendency of the ventricles to fibrillate persisted even after the second slowing phase began, so that ventricular fibrillation frequently terminated a burst of tachycardia after an arrest of fifteen seconds or more. Because of this extension of the period during which ventricular fibrillation might be expected, 8 of 10 animals given calcium gluconate-idonate, and 7 of 8 given calcium gluconate died of fibrillation, compared with 6 of 13 given calcium chloride.

Appreciably larger quantities of calcium as gluconate-idonate or as gluconate are required to produce death than are needed when calcium is given as the chloride. This is reflected in the terminal serum calcium values, the means of those for both organic salts being significantly higher than that with the chloride. The gluconate-idonate and the gluconate do not appear to differ appreciably in their effects. It is possible that the difference between the chloride and the sugar acid salts can be explained in part by the different amounts of calcium ions they yield.

SUMMARY

Calcium chloride, whether given orally to rats or intravenously to dogs, is definitely more toxic than calcium gluconate-idonate or calcium gluconate.

Calcium gluconate-idonate and calcium gluconate differ very little in their toxicity for dogs and rats. Over a long period of time (seventy days) calcium gluconate proved lethal to 2 of 10 rats, and calcium gluconate-idonate to none of 10.

Intravenous injections of 0.205 M solutions continued to the point of cardiac arrest (usually with ventricular fibrillation) revealed no differences between the effects of gluconate and calcium gluconate-idonate.

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FATAL RENAL INSUFFICIENCY FOLLOWING THE ADMINISTRATION OF SULFAPYRIDINE*

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THE object of this paper is to describe briefly a case of fatal renal insufficiency accompanied by hematuria and oliguria following the clinical use of sulfapyridine.

CASE REPORT

Clinical History. L. P., an 80-year-old white spinster, was admitted to University Hospitals on April 29, 1939, with a history of an upper respiratory infection of eight days' duration and a severe productive cough of three days' duration. Her past history was essentially negative except for pneumonia three years previously.

Physical Examination. Her temperature was 102.9° F., her pulse 80, her respirations 28, and her blood pressure 165 systolic and 85 diastolic. She was a well-developed and well-nourished woman, looking much younger than her stated age. Her throat was red and a slight mucopurulent discharge was present in the pharynx. There was impairment of the percussion note at the bases of both lungs posteriorly, together with numerous coarse and fine crepitant râles. A systolic murmur was noted at the apex. The rest of the physical examination was negative.

Laboratory Findings. The urine on admission was entirely negative. The leucocyte count was 14,600, erythrocytes 4.1 million, and hemoglobin 80 per cent Sahli. The Kline exclusion test was negative. Blood cultures gave no growth. The blood urea nitrogen on admission was 15 mg. per 100 c.c. Sputum typing yielded a type XI pneumococcus. X-ray examination of the chest showed what was interpreted as bronchopneumonic infiltration in both lower lobes.

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The patient was given 4 Gm. of sulfapyridine during her first hospital day (April 30). The following morning her temperature was normal and the drug was discontinued. While her urinary output at this time was not measured accurately, it appeared to be adequate.

On May 2, the third hospital day, the temperature rose to 100.4° F. and the administration of sulfapyridine was resumed. During the next thirty-six hours the patient received a total of 4 Gm. in 0.5 Gm. doses. The drug was then discontinued.

On May 3 the temperature fell to normal, the pulse was 80, and the respirations 22. On this day, however, the patient complained for the first time of abdominal pain; she was unable to void. The next day 45 c.c. of grossly bloody urine were obtained by catheter. The blood sulfapyridine was 1.8 mg. per 100 c.c., and the blood urea nitrogen was 30 mg. per 100 c.c.

On May 5 the total output of urine was only 6 c.c. This was bloody and was obtained by catheter. The blood urea nitrogen had increased to 42 mg. per 100 c.c. Despite this her pneumonia showed signs of resolution. One intravenous infusion of 300 c.c. of physiologic saline and 100 c.c. of 50 per cent sucrose were given.

During the next three days (May 6, 7, and 8) only 15 c.c., 2 c.c., and 86 c.c. of urine, respectively, were obtained by catheter. All the specimens were grossly bloody and contained large traces of albumin. Specific gravities corrected for protein varied from 1.010 to 1.019. The patient received several infusions of physiologic saline and glucose. On May 6 the blood pressure, which had previously been about 165 systolic and 80 diastolic, rose to 190 systolic and 90 diastolic.

The patient died of uremia on May 8, the ninth hospital day and five days after the administration of sulfapyridine had been discontinued. She showed no convulsions. The blood urea nitrogen on the day of her death was 104 mg. per 100 c.c. The final clinical diagnoses were toxic glomerulonephritis due to sulfapyridine and uremia.

Autopsy was performed two hours after death. It was limited to the abdomen, but examination of the thoracic organs through the abdominal incision revealed that the pneumonia was either completely resolved or minimal. The heart appeared to be enlarged.

The essential pathologic findings were in the urinary tract. Each kidney weighed 200 Gm. and was the seat of slight arterial and arteriolar vascular disease. Cross section showed marked bulging of the parenchyma, which was pale gray in color; the usual striations in the cortex were obscured. The pelves were of normal size and shape but were lined by a thick, edematous, grayish-brown mucosa which showed irregular hemorrhages extending into the adjacent peripelvic fat. The mucosa of the minor calices and of the renal papillae was greenish-brown and necrotic and presented a ragged surface covered by deposits of granular and amorphous reddish-brown debris. Chemical analysis of this material for sulfapyridine and its derivatives was negative.

The ureters showed no dilatation. In their upper portions the mucosa was thickened and showed many small areas of hemorrhage. However, the distal 4 cm. of each ureter, including the portions traversing the wall of the bladder, revealed a swollen and necrotic, greenish-brown mucosa, and the lumina were markedly reduced. The bladder showed diffuse edema and focal hemorrhages of the mucosa.

Microscopically, in addition to slight arteriolar sclerosis, the kidneys were the seat of a marked nephrosis. The epithelial cells of the tubules were enlarged and presented a pale-staining, foamy, or transparent cytoplasm, and very distinct cell borders. The nuclei were of usual size and showed no necrotic change. The swelling of the cells had resulted in moderate diminution of the lumina of the tubules. The glomeruli showed no significant change. There was no inflammatory exudate.

Microscopically the renal pelves showed marked edema of the mucosa, foci of polymorphonuclear and lymphocytic infiltration, and focal hemorrhages extending into the adjacent peripelvic fat. The lining of the minor calices and of the renal papillae was necrotic and ulcerated, and partly replaced by masses of blue and greenish-brown necrotic material. This lesion was associated with dense infiltrations of polymorphonuclear cells. The upper portions of the ureters showed a slight degree of inflammation, edema, and focal hemorrhage, while

the terminal portions extending to the orifices in the urinary bladder showed the same type of necrotizing and suppurative inflammation present in the renal calices. The urinary bladder was the seat of an acute hemorrhagic cystitis and showed polymorphonuclear and lymphocytic infiltration of the mucosa.

The liver, spleen, and gastrointestinal tract showed no significant gross or microscopic change.

The final pathologic diagnoses were: acute necrotizing and purulent pyelitis and ureteritis with hemorrhage; marked acute nephrosis; acute hemorrhagic cystitis; minimal arterial and arteriolar nephrosclerosis.

COMMENT

Although this patient was admitted to the hospital with a bilateral bronchopneumonia, she did not appear to be seriously ill. On admission her urine was negative, her urinary output was satisfactory, and her blood urea nitrogen was only 15 mg. per 100 c.c. In the treatment of her pneumonia she received a total of 8 Gm. of sulfapyridine, of which 4 Gm. were given on her first hospital day and 4 Gm. on her third and fourth hospital days. She was apparently making an uneventful recovery until the fourth hospital day, when she complained for the first time of abdominal pain and inability to void. Catheterization the following day yielded 45 c.c. of grossly bloody urine. During the next four days, although her pneumonia appeared to be resolving, she did not void spontaneously, and a total of only 109 c.c. of bloody urine were obtained by catheter. Blood urea nitrogen determinations were 30 mg. per 100 c.c. on May 2, 42 mg. on May 5, and 104 mg. on May 8, the day of death. Death occurred eight days after sulfapyridine therapy was started.

At autopsy the essential pathologic findings consisted of an acute necrotizing, purulent, and hemorrhagic pyelitis and ureteritis, and a marked acute nephrosis. The inflammatory lesion was especially severe in the minor calices and renal papillae and in the terminal portions of the ureters which were obstructed as a result of mucosal swelling, necrosis, and exudate. The lesions appeared to be of short duration; there was no evidence of chronicity. Although no calculi were found in the urinary tract, there were extensive deposits of reddish-brown granular material lining the pelvic surface of all the renal papillae. Chemical analysis of this material for sulfapyridine and its acetyl derivative was negative. There was no inflammation within the kidney.

Similar lesions of the urinary tract associated with the formation of uroliths, consisting of the acetyl derivative of sulfapyridine, have been produced experimentally in animals by means of the oral administration of the drug.[1, 2] Antopol and Robinson[1] noted the formation in the urinary tracts of susceptible rats, rabbits, and monkeys of aggregates of needlelike crystals, especially in the ureters and most frequently at the level of the bony pelvic brim. These lesions were often associated with more or less complete urinary obstruction. The early picture in the urinary tract was that of calculous ureteritis and pyelitis; with involvement of the kidney a pyelonephritis ensued.

Hematuria has also been reported as a complication of sulfapyridine therapy in man. Southworth and Cooke[3] recently described three such patients, two of whom had severe renal and ureteral pain, while two had nitrogen retention.

All recovered promptly when the drug was stopped and adequate fluids were given. From the experimental and clinical evidence available thus far, it would appear that sulfapyridine is capable of producing a hemorrhagic and suppurative inflammation of the urinary tract, especially of the ureters and renal pelves, associated with necrosis and formation of concretions. The latter consist of sulfapyridine and its acetyl derivative. Possibly the concretions are formed first, and the inflammatory lesions are secondary. The attendant oliguria and pain are presumably due to the obstruction and urinary stasis in the pelves or ureters caused by the inflammation, the calculi, or both these factors.

Both the clinical course and the autopsy findings in the present case justify the belief that the sulfapyridine was the etiologic agent responsible for the urinary tract lesions. Prior to the use of the drug the patient's urine was negative and her urinary output was adequate. Following the administration of 8 Gm. of sulfapyridine there was rapid development of hematuria, oliguria, and azotemia. Except for small doses of codeine, no other drugs were given. The patient received one infusion of 100 c.c. of 50 per cent sucrose, but this was administered after the onset of the oliguria and hematuria. The necrotizing and hemorrhagic type of lesion in the pelves and ureters indicates a toxic and irritative injurious agent of chemical rather than bacterial nature.

The marked tubular disease present in the kidneys may be due largely, or in part, to the sucrose which the patient received shortly before death, rather than to the sulfapyridine. Nephrosis has been observed at autopsy in patients who received sucrose during life.[4] Similar tubular damage has also been produced in animals by the intravenous administration of sucrose, although the amounts used were comparatively large and the nephrosis was not associated with renal insufficiency or with inflammation in the urinary tract.[5] That the sucrose is not the primary injurious agent in this case is indicated conclusively by the fact that it was used after the onset of hematuria and oliguria. The patient's pneumonia was minimal and in the process of resolution and was in all probability not related to the fatal outcome or to the disease in the urinary tract.

SUMMARY

A case of fatal renal insufficiency incident to the administration of sulfapyridine is described. The patient was an 80-year-old woman who received a total of 8 Gm. of the drug over a period of three days in the treatment of a bronchopneumonia. She then rapidly developed abdominal pain, hematuria, and marked oliguria. During the next five days a total of only 154 c.c. of bloody urine was obtained by catheter. Death occurred in uremia eight days after the onset of administration of the sulfapyridine and five days after it was discontinued. At autopsy there was a severe necrotizing, suppurative, and hemorrhagic ureteritis and pyelitis, with obstruction of the terminal portions of both ureters, and a marked acute nephrosis.

Grateful acknowledgment is made of aid rendered by Dr. Joseph M. Hayman, Jr.

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THE EFFECTS OF INSULIN ON SERUM LIPIDS AND CHOLINE ESTERASE IN SCHIZOPHRENIA*

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ALTHOUGH we were unable to demonstrate any consistent immediate effect of insulin on the postabsorptive level of the whole blood lipids in a previous study,[12] we observed a mild lipemia on repeated treatments. After cessation of treatment the lipids remained elevated in those patients showing remission but tended to return toward the initial level in the unimproved patients. Since serum lipids are more susceptible to change under the influence of extraneous agents than are whole blood lipids, we studied the immediate effects of insulin on the postabsorptive level of serum lipids as well as the chronic effects of repeated doses in another series of patients. Hematocrits were included to control variations in serum concentration, an important factor in insulin coma because of the severe perspiration. Since insulin has been suspected of exerting a stimulating effect on the sympathetic nervous system[5] and since it has been postulated that the choline esterase activity of serum is related to the autonomic balance,[7] we have included this variable as a corollary to the study of lipid variation coincident with variation of autonomic activity.

METHODS

The prolonged effects of the insulin hypoglycemia treatment on serum lipid, choline esterase, and hematocrit were studied in 17 schizophrenic patients. Two blood samples (a week apart) were taken before beginning treatment, another during the fourth week of treatment, and two more (a week apart) after cessation of treatment. In order to study the immediate effect of insulin, further samples were taken on 12 patients (in the fourth week of treatment) in the postabsorptive state, during the period of coma, and one hour after the administration of sugar which was used to terminate the coma. Serum phospholipid and total lipid were determined in duplicate by Bloor's oxidimetric methods.[2] Free and total cholesterol were determined by Turner's modification of the Okey digitonide method.[17] Choline esterase activity was determined by Ammon's gasometric method.[1] The relative volume of serum to cells was determined after centrifuging the heparinized blood in a graduated tube.

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RESULTS

In Table I are presented the means and criteria of significance of differences of serum lipids, choline esterase, and hematocrit obtained before treatment, during the fourth week of treatment, and after cessation of treatment. It is apparent that the various lipid fractions and choline esterase reach the maximum values during the midcourse of treatment. With the exception of free cholesterol the mean values are significantly lower at the end of treatment than those in midcourse, but they are significantly higher than the pretreatment means.* It is noteworthy that the hematocrit value did not change significantly during the course of treatment.

TABLE I

SHOWING THE DIPHASIC COURSE OF SERUM LIPIDS AND CHOLINE ESTERASE IN CONTINUOUS INSULIN TREATMENT*

VARIABLE	N	MEAN VALUES			SIGNIFICANCE OF DIFFERENCE IN TERMS OF THE "T" CRITERION		
		BEFORE TREATMENT	IN MIDCOURSE OF TREATMENT	AFTER TREATMENT	BEFORE TREATMENT VS. MIDTREATMENT	MIDTREATMENT VS. AFTER TREATMENT	BEFORE TREATMENT VS. AFTER TREATMENT
Total lipid mg./100 c.c.	17	402.7	546.2	449.6	4.9*	3.1*	2.96*
Phospholipid mg./100 c.c.	17	177.4	231.6	202.3	4.6*	2.5†	5.08*
Total cholesterol mg./100 c.c.	17	159.8	192.5	173.8	4.7*	3.0*	2.69†
Free cholesterol mg./100 c.c.	17	49.1	54.9	46.5	1.8	3.1*	0.46
Choline esterase c.mm./hr./c.c.	14	68.2	82.1	80.0	6.04*	1.17	3.04*
Hematocrit per cent	17	44.4	44.4	44.2	--	--	--

*$P < 0.01$.
†$0.05 > P > 0.01$.

The behavior of serum lipids and choline esterase in the midcourse of treatment was exceptionally uniform from individual to individual. The rise in lipid level occurred in all cases, and choline esterase rose in all cases except one, in which there was no change. There occurred considerable variation among different patients in the values obtained during the two weeks following the treatment. These data were discussed in a separate paper[13] in which it was shown that the serum lipids remained elevated in those patients who improved coincident with the treatment but returned toward the pretreatment level in those patients who remained unchanged mentally.

In Table II are presented the means and criteria of significance of differences for serum lipids, choline esterase, and hematocrit in 12 patients before and during the course of a single insulin treatment. Although for all the variables we find a rise in the mean level above the postabsorptive level and a tendency to drop again after the administrations of sugar, only the trends for choline esterase and hematocrit are statistically significant. The behavior of lipids during coma, and especially on administration of sugar, varied greatly

*The significance of differences was determined through "t" criteria.

from individual to individual. In 8 of the patients the lipids rose to greatly varying degrees, while in 4 patients there was a fall or no change.

All patients except one showed a rise in choline esterase and all showed the increase in hematocrit during coma. The question arises whether or not the increased blood concentration accounts for all the increase in choline esterase. The intra-individual correlation coefficient, obtained on the control and coma values of hematocrit and for choline esterase, was 0.67. Thus the hematocrit change accounted for 26 per cent of the variation of choline esterase. The larger proportion of the variation in choline esterase may, therefore, be ascribed to factors other than hemoconcentration. Since the mean hematocrit change was 5.8 per cent and that for choline esterase was 12.5 per cent, we believe that insulin produced a significant rise in choline esterase.

TABLE II

SHOWING MEAN CHANGES IN SERUM LIPIDS, CHOLINE ESTERASE AND HEMATOCRIT DURING INSULIN COMA AND AFTER ADMINISTRATION OF SUGAR

VARIABLE	N	MEAN VALUES			SIGNIFICANCE OF DIFFERENCES IN TERMS OF THE "T" CRITERION
		CONTROL	COMA	AFTER SUGAR	CONTROL MEAN VS. COMA MEAN
Total lipid mg./100 c.c.	12	554.6	599.2	585.9	2.37
Phospholipid mg./100 c.c.	12	229.0	237.0	228.4	1.16
Total cholesterol mg./100 c.c.	12	192.3	203.9	191.1	2.19
Free cholesterol mg./100 c.c.	12	55.7	58.0	57.3	1.46
Choline esterase c.mm./hr./c.c.	10	84.8	95.5	87.7	4.30*
Hematocrit per cent	12	44.43	47.09	46.60	5.99*

*$P < 0.01$.

DISCUSSION

The variable response of the postabsorptive level of the serum lipids among different individuals indicates that insulin acts only indirectly on the serum lipids, perhaps through a disturbance in the carbohydrate or acid-base balance. Reports of other workers on the effect of insulin on the postabsorptive level of blood lipids are equally inconsistent. In normal men Christomanos[4] reported a slight fall, while Bruger and Mosenthal[3] found no consistent effect. In animals a drop in blood lipids was reported by Page, Pasternak, and Burt,[11] Schmidt and Ssaatchian,[15] Himwich and Spiers,[6] and Wertheimer;[18] no change was indicated by the results of Rony and Ching[14] and Miller;[10] and a rise was found by White.[19]

There is little reason for doubting that insulin lowers an abnormally high blood lipid level, although this is likely to be due to an indirect effect of insulin on carbohydrate metabolism. Treatment of the lipemic diabetic person with insulin lowers the blood lipid in both human subjects[8] and dogs.[9] Insulin causes a phlorizin lipemia[18] or nephrotic lipemia[16] to disappear. On the other hand, we have found a prolonged rise in serum lipids after insulin administration in patients who have an abnormally low serum lipid level.[13] The daily injection

of large doses of insulin appears to raise the lipids toward the normal level, which is maintained after cessation of treatment only in those patients who improve mentally coincident with the treatment. The mean choline esterase level of the patients did not differ significantly from that of normal subjects. The insulin therapy produced a rise in choline esterase in all patients, followed by a return toward the initial level without regard to the clinical progress of the patients.

SUMMARY

During the course of treatment of 17 schizophrenic patients with large daily doses of insulin, the serum phospholipid, total cholesterol, free cholesterol, total lipid, and choline esterase rose above the pretreatment level. These values remained elevated for two weeks following treatment.

In 12 patients studied during the fourth week of treatment insulin did not produce any consistent immediate change in serum lipids, but it increased the choline esterase to a significantly greater extent than could be accounted for by hemoconcentration.

I am indebted to Mr. E. B. Romanoff for some of the choline esterase determinations.

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VARIATION OF BLOOD PRESSURE WITH BRIEF VOLUNTARY MUSCULAR CONTRACTIONS*

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IN A PREVIOUS study[2, 3] the blood pressure of various subjects was taken from time to time during periods of from one to two hours, while graphic records were made concerning their states of skeletal muscle tension.† Determinations were made of systolic as well as of diastolic pressure in some patients with vascular hypertension as well as in subjects of normal type. Skeletal muscle tension was measured in terms of action potentials, employing the right biceps-brachial muscle group in all instances, along with one or more other groups in most instances. In order to eliminate the influence of change of posture to the lying position, no reading of blood pressure was considered until the subject had been lying for at least fifteen minutes.

The evidence pointed clearly to a relationship in man between the state of contraction in the manifold muscle groups of the individual and his blood pressure at a corresponding instant. This accords with an extensive literature on physical exercise, which it seems unnecessary to review here, but from which it is well known that during vigorous muscular contraction, the blood pressure rises, at least until fatigue sets in; this applies to systolic, if not always to diastolic, pressure. However, one writer (Fishberg,[4] 1934) finds that slight muscular exercise produces little or no change in the blood pressure. In any event, the relationship between contraction in divers skeletal muscle groups and variations in blood pressure is not simple, and is readily obscured in the presence of other factors that influence the blood pressure. More evidence is needed to confirm the existence of this correlation and to clear up our conceptions about it.

The impression has been gained that under conditions kept fairly constant otherwise, a marked change in the state of general muscle tension is likely to be accompanied by a shift in the blood pressure level. Blood pressure tends to fall with progressive muscular relaxation, especially as this nears completion; but after lower levels of pressure are once established, merely prolonging the rest, without increasing the degree or extent of relaxation, does not necessarily effect an additional fall in pressure. Likewise, if from the outset there is prolonged tension in the muscle groups studied during

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†By tension is here meant active contraction, not passive stretch.

the rest period, there is not necessarily a marked rise of pressure; if general muscle tension fluctuates fairly constantly within certain limits, the blood pressure may show little variation during the period.

While it is certain that skeletal muscles do not necessarily relax fully during a period when an individual attempts to rest,[5] this is not generally realized as yet, and there is still the tendency in many quarters to identify lying down with relaxation. The influence of posture can be eliminated most effectively if, while keeping this constant during an experimental period, we produce alternately contraction and relaxation in selected muscle groups, and determine whether there occur corresponding increases or decreases in blood pressure. To effect this in the present studies, the subject, while lying at rest in other respects, clenches his right fist for several minutes, generally about five, sometimes up to ten.

Although the studies have been made chiefly in the lying position, similar results are observed if the subject sits up in a fairly comfortable chair. In that position a certain degree and extent of relaxation commonly occurs under normal conditions, as has previously been shown by various methods. For example, the knee jerk, responding to an intermittent stimulus of constant magnitude, shows a characteristic decline in extent in many instances.[6] Furthermore, an individual may be seated but so relaxed that a continuous record of zero activity, or nearly so, is shown from the muscles of either arm. In short, while sitting, relaxation may be more or less complete in muscle groups whose activity is not required for that posture.

As stated previously, preliminary studies of several hypertensive patients in the sitting posture indicated that the blood pressure often rose when they contracted the muscles in a part (such as the upper arm) during a test period of about ten to sixty seconds, falling subsequently if they then relaxed those same muscles while remaining in the sitting posture. The customary method of combined palpation and auscultation was employed. Such procedures in studying rise and fall of pressure obviously enable us to rule out the influence of posture, since this continues constant; they also keep emotional factors down to a minimum of change.

Observations can be made more precisely if muscle tensions are measured in terms of action potentials. Two amplifier string galvanometer assemblies were employed, recording action potentials, as stated previously, to a fraction of a microvolt. For this, 15 subjects were available. The blood pressure was normal in 11, 6 of whom had received training to relax, while the other 5 were athletes. The 4 remaining subjects showed chronic high blood pressure and were being trained to relax. All subjects had participated in previous experiments, and accordingly, were well accustomed to the conditions of test. In the following studies, the subject, as a rule, reclines and has been instructed to clench his fist continually and vigorously, beginning when he hears a telegraph key click near his ear. The moment of this click is indicated on the photographic records by the movement of the pointers of two Duprez signal magnets —one placed before each camera. A second click recorded similarly is the

signal to cease a requested muscular contraction, such as clenching. Accordingly, the time relations can be charted accurately. In order to eliminate subjective errors in determining blood pressure, the Tycos self-recording sphygmomanometer is again employed in all but the early records in this series. As said previously, the point of systolic pressure is sharply indicated on the charts made with this instrument, but there is question at times concerning the reading of the diastolic pressure. Accordingly, we may believe that the figures for systolic pressure have a negligible error, but no such assumption is made regarding those for diastolic pressure. However, since all readings were made by two persons in association, we may assume that errors, if any, were in the same direction, and that the results for diastolic pressure are satisfactory at least for purposes of comparison. In any event, the general character of the results on diastolic, as well as on systolic, pressure does not seem to differ very much whether the automatic apparatus is used or the ordinary method of palpation plus auscultation.

To determine and evaluate the influence on blood pressure of the voluntary contraction of selected muscle groups, it is necessary to keep other factors as nearly as possible constant. Accordingly, in the present experiments, noises and other disturbing factors are avoided. It was evident that the subjects were not in an emotional state. The instruction to contract certain muscle groups, while keeping others as relaxed as possible, was probably carried out more precisely by the subjects trained to relax; but the athletes were skillful at this as well.

While the results to be presented are not positive in all instances, they seem to reveal a trend distinctly. The type of result often secured in a subject with normal blood pressure upon prolonged complete or approximately complete relaxation interrupted at intervals by muscular contractions, such as clenching the right fist, appears in Fig. 1.

Ro. was a young woman, 24 years old, who had recovered from a condition of severe nervous depression, pari passu as she learned to be relaxed. She readily achieves marked relaxation in the right arm (biceps brachialis) and the left leg (quadriceps femoris) muscles in which potentials are measured; the voltages generally are near 0 microvolt, but do not exceed 1 microvolt. She is somewhat less successful in relaxing the abdominal muscles, and still less those of the eyes. On the whole, the degree of relaxation attained is sufficient for present purposes.

About fifteen minutes after lying down, the first blood pressure is taken, whereupon recording of action potentials is begun, while she relaxes fairly for about fifteen minutes more. Upon firmly clenching the fingers of the right hand, there occurs promptly an increase in systolic pressure (8 mm.) and in diastolic pressure (5 mm.). In this and in similar figures considered here, the blood pressure readings during any contraction will be set down as positive or negative, as compared with the last reading before the contraction. (An alternative procedure, but leading to practically similar results, would be to take the mean of the readings prior to the contraction.) This increase is not maintained in the second blood pressure reading toward the close of the five-minute period of clenching, where the values are but + 4/-1, as compared with the reading prior to the contraction. During this same five-minute interval, action potentials from the right arm muscles decline somewhat in voltage, showing that the patient has not mtaintained the clenching of the fist steadily; possibly this explains in part

the decline in pressure during the same period. Upon clenching the second time, the rise in pressure is more marked than before (12/8), while the second reading toward the close of the five-minute period of clenching is 12/16. Evidently, there is no decline in pressure during the five-minute period of clenching this time, possibly because the abdominal muscles contracted somewhat more during this period, producing an additive effect on the pressure.

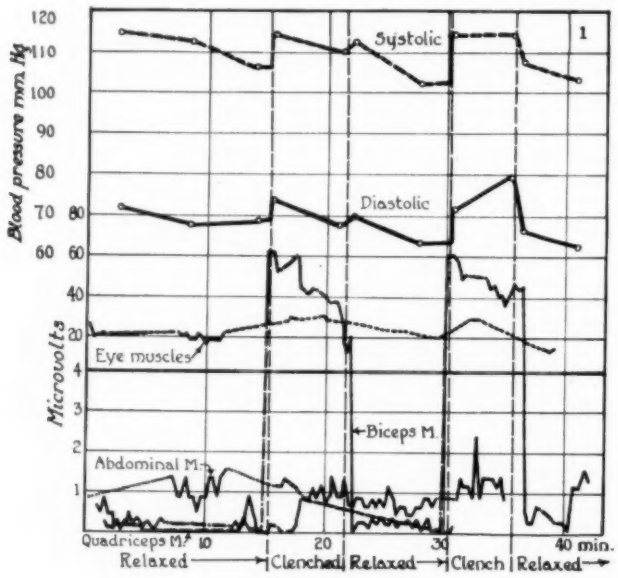

Fig. 1.—Showing rise of systolic and diastolic pressures during clenching of the right fist in a woman aged 24, trained to relax fairly. Plotted against time are systolic and diastolic blood pressures in millimeters of mercury, but also microvoltage of action potentials from certain muscles in the right arm, left thigh, abdomen, and about the eyes (perhaps including retinal potentials).

As described previously, the cameras do not operate continuously and, in consequence, records of action potentials are not complete for four leads. Accordingly, interpolation appears on this graph as well as in Figs. 3, 4, and 5.

In the 11 subjects with normal pressure, 70 determinations in all were made during clenching of the right fist. Systolic pressure was increased in 64, and diastolic pressure was increased in 63 of these determinations.

A composite graph for these 70 determinations appears in Fig. 2, covering a time period of ten minutes. During clenching of the right fist in these subjects there occurs, on the average, a significant rise in systolic and diastolic pressures up to at least ten minutes. There is some indication of a tendency for diastolic pressure to accompany systolic pressure in its rises. (In most instances clenching was not prolonged after about five minutes.) In all instances readings were not made each minute, since inflating the cuff so often would inevitably influence the results. Accordingly, no conclusions are warranted from the character of the curve shown in Fig. 2, except that, in general, the results are strongly positive.

Fig. 3 furnishes an illustration of a patient suffering from vascular hypertension of advanced type.

L., a matron of about 37 years, was referred for study from the Billings Hospital through the kindly cooperation of Doctors Franklin McLean and Louis Leiter. The diagnosis

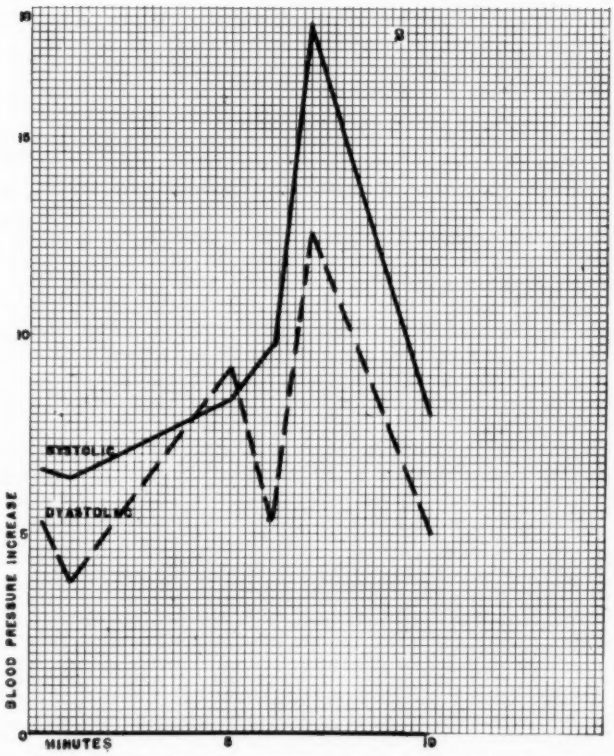

Fig. 2.—Rise in systolic and in diastolic pressure during protracted clenching of the right fist. Composite graph of 70 determinations in 11 subjects with normal pressure.

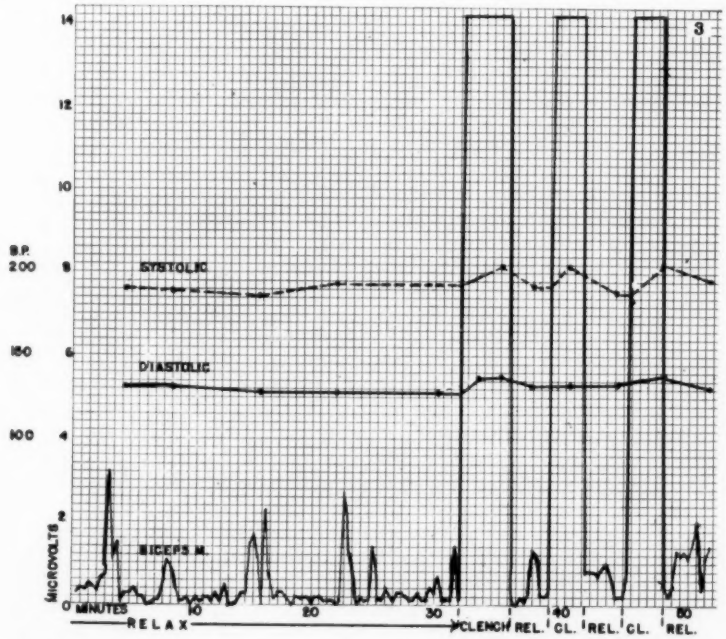

Fig. 3.—Plotted against time are systolic and diastolic blood pressures, as well as action potentials from the flexors in the right upper arm. Rises in systolic and diastolic pressures generally mark the intervals in which she clenches her right fist as contrasted with the periods in which she does not.

was hypertension, with evidences of advanced renal pathology. Action potentials from the flexor muscles located in the right upper arm are plotted against time. As will be noted, her relaxation is far from perfect. After about two minutes of clenching the right fist, pressure rose in the amount of 5/9, and about three minutes later the increase became 11/10. During the five-minute period, when clenching ceases and relative rest is resumed, the pressure drops back approximately to the levels which preceded the act of clenching; but two minutes after clenching has been resumed once more, pressure again increases in the amount of 11/1. Decline of pressure occurs again during the following control period. During the third test period of clenching, the pressure rise after about four minutes is 17/5. Thereafter, the pressure declines again approximately to former resting levels.

The data on four subjects with hypertension are too few to graph, but the results are roughly on the same order as for those with normal pressure. At one minute the average rise is 5.7/4.3; at five minutes it is 8.3/5.7; at ten minutes it is 9.7/8.0. Upon voluntary contractions, while lying, we should expect greater increase in blood pressure—in millimeters of mercury—during states of chronic high blood pressure than during normal states. Our findings, however, show the greatest increases, as a rule, in three subjects whose pressure levels are normal. The explanation probably is that these were athletes: they obviously clenched their fists more tightly and vigorously than did the subjects in the other groups. This observation is readily confirmed upon reading the voltages from muscles in the arm during clenching, which are found to exceed considerably the similarly recorded voltages in the trained subjects (not athletes) and in the subjects with abnormally increased blood pressure. It seems safe to conclude that the more vigorous contraction in the normal athletes accounts for the greater increase in pressure observed in the group of athletes as compared with the hypertensive group.

In all subjects more strongly positive results are often secured if additional muscle groups participate in the voluntary contraction. This is well illustrated in Fig. 4.

The subject is a professor in a medical department, aged 37 years, whose original complaints were from spastic colitis of thirteen years' duration; there were no symptoms of cardiovascular origin. At the time this record is taken recovery was evidently complete, and his technique at relaxation, as shown in Fig. 4, was adequate. After lying down for about twenty-five minutes, the pressure reading was 118/69. Upon clenching his right fist vigorously, the pressure taken at once was 122/76, an increase of 4/7. When clenching had been protracted for five minutes, the reading became 136/81, a total increase of 18/12. Upon stiffening both legs, but also continuing to clench the fist, the pressure taken at once was 145/86, a total increase of 27/17; when protracting these contractions for five minutes, the reading became 147/87, a total increase of 29/18. When instructed to relax once more, the reading fell at once to 127/74—a return approximately to the levels present prior to the original clenching. From this level, subsequent clenching of the right fist produces a rise in systolic and in diastolic pressures to a peak increase of 11/9—a figure less than half of the peak increase noted during the joint contraction of right arm and both leg muscles, namely, 29/18.

Augmentation of blood pressure in the lying position occurring upon increased involvement of skeletal muscle groups (progressive muscular tension) is further illustrated in Fig. 5.

An athlete, aged about 20 years, shows normal pressure. After lying quietly for almost fifteen minutes, the record begins with right arm muscles recorded relaxed perfectly, while

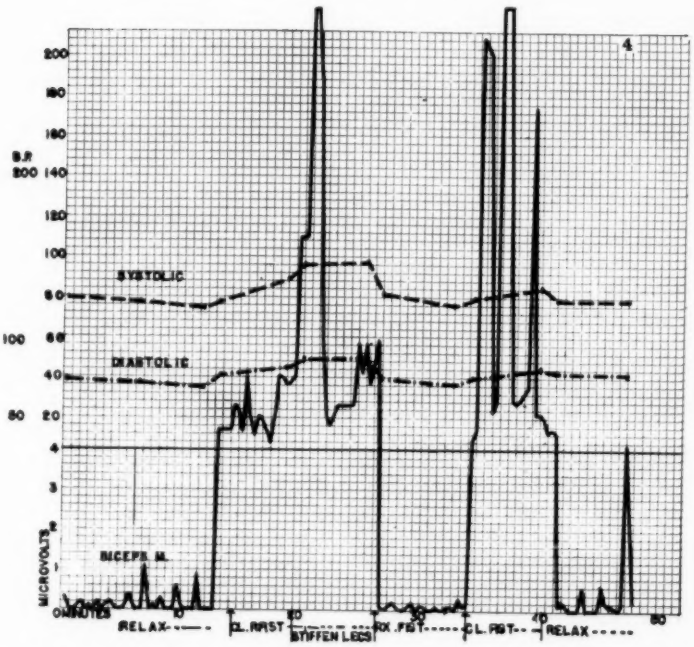

Fig. 4.—Systolic and diastolic pressures, as well as action potentials from the flexor muscles in the right upper arm, plotted against time. Subject is trained to relax; blood pressure is normal. The rise in pressure occurring upon clenching the right fist is augmented, both systolic and diastolic, upon additional voluntary contraction in both legs.

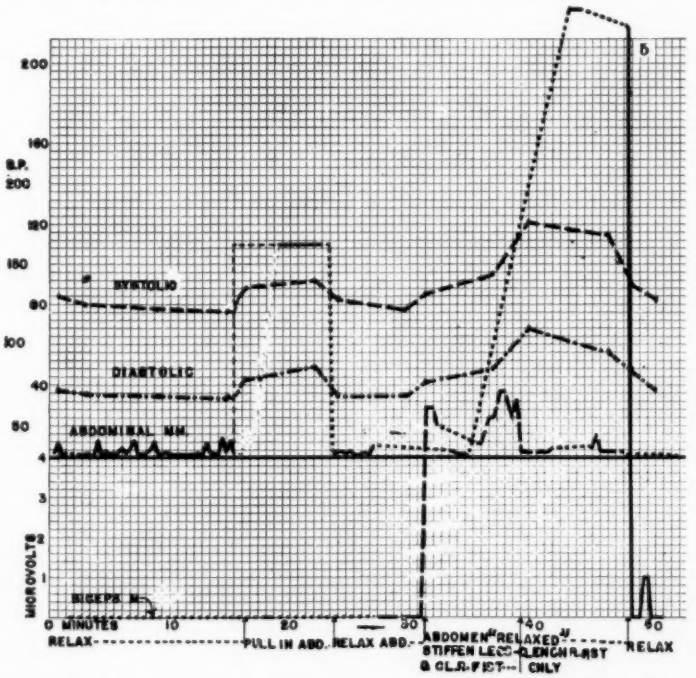

Fig. 5.—Plotted against time are systolic and diastolic blood pressures, as well as action potentials from the flexor muscles in the right upper arm and from abdominal muscles. A sustained rise in systolic and diastolic pressures occurs with continued contraction in the abdominal group. Pressure returns to resting levels upon muscular relaxation. When the right fist is clenched and the legs stiffened as well, the pressure rise is very definite; but it becomes greatly augmented when, contrary to instruction, the abdominal muscles are contracted in addition. Upon relaxation, pressure returns to former resting levels.

the electrodes in abdominal muscles reveal over 4 microvolts of potential differences. The pressure rises from 120/67 to 135/78 upon "pulling in" the abdominal muscles, and at the end of five minutes (while the arm muscles continue to be relaxed) the pressure reaches 140/86—a total rise of 20/19. Upon relaxing the abdomen to former levels, a fall occurs promptly to 128/68, and after five minutes of continued relaxation, the blood pressure has returned to former resting levels, 122/68. The instruction to clench the right fist and to stiffen both legs is followed promptly by a rise to 131/77, and when this is continued for five minutes, the pressure increases to 144/85, a total increase of 22/17. In addition, but contrary to instruction, he pulls in the abdominal muscles, whereupon the pressure rises to 176/110, a total increase of 54/42. While these various muscle groups continue in sustained contraction, there occurs at the end of five minutes a fall to 168/95, reducing the total increase at this point to 46/27. Upon instruction, the abdominal muscles now relax perfectly for the first time in this record, but the right arm remains somewhat tense; thereupon blood pressure falls to 137/84, and after five minutes to 128/72, which is 8/5 higher than before the original voluntary contraction of the abdominal muscles.

Increased blood pressure following upon "pulling in" the abdominal muscles may be due, in part at least, to pressure upon abdominal (splanchnic) blood vessels. Evidence to this effect will be considered in a later article. However, this is probably not the full explanation, for little or no such pressure on the abdominal blood vessels occurs if the subject is instructed to make the abdominal muscles rigid but without squeezing inwards, or if he is instructed to push out the abdominal wall by muscular effort; yet these alternatives are accompanied by rise of blood pressure in various instances recorded during the present studies.

When the subject, lying down, engages in a voluntary contraction following a period of attempted relaxation, a rise of pressure is not always clearly notable by ordinary methods of measurement. Sometimes the reasons for such negative results are obscure; at other times the interpretation seems fairly evident. A negative instance with the subject L., which can be compared with Fig. 3, follows:

She fails to relax when requested to do so. Action potentials from the right arm muscles are relatively very high before as well as after she clenches her fist, frequently and repeatedly reaching 7 microvolts or more. Toward the end of the initial period of unsuccessful attempts to relax, the pressure is 208/120. Not an increase, but a drop to 197/118 occurs after five minutes, during which she has been clenching her right fist. After five minutes more of this activity, the pressure has risen to 213/122. She ceases to clench her fist, and returns once more to attempts to relax which prove unsuccessful; after intervals of five and eighteen minutes, the readings are 219/119 and 210/118, respectively. In short, blood pressure is not higher during the period of clenching than during the period of attempted relaxation, apparently because she fails to relax. In the relaxing periods shown in Fig. 3, she was considerably more successful.

Accordingly, this instance illustrates once more that the attempt to relax, if unsuccessful, does not characteristically bring with it a fall of blood pressure, as compared with a foregoing period during which contraction has been marked.

Evidently, if a muscle group has been in a state of marked contraction, a moderate additional contraction therein may be insufficient to make itself apparent in a change in the recorded blood pressure. Presumably it is the relative degree of augmentation in contraction (not the absolute amount, measured in dynes, calories, or microvolts) which shows up in the results.

A generalization here, if data were sufficient to permit this, could be expressed in terms analogous to Du Bois-Reymond's law applied in the neuromuscular field. This accords also with the comments made previously that after contraction has subsided so that it is minimal, augmentations in contraction are accompanied by more obvious rises in blood pressure.

SUMMARY AND CONCLUSIONS

Experiments are conducted on 11 subjects with normal blood pressure and on 4 with chronically high blood pressure, all of whom relax at least fairly well upon request, whether through special training or otherwise. Electrical measurements are taken of contraction (action potentials) in various muscle groups, while blood pressure is recorded at certain times from the left upper arm.

In an individual otherwise relaxed, successive contraction of a muscle group or groups (for example, clenching one fist) for periods of several minutes or more, and then relaxing completely for similar periods, is frequently accompanied by corresponding rises and falls of systolic and diastolic pressures. The extent of the relative rise and fall depends not only upon the intensity of the particular voluntary contraction but also upon the degree and extent of relaxation present in the general musculature. No contrasting rise in pressure may be evidenced if the patient is not well relaxed before he clenches his fist. The addition of marked contraction in other regions (e.g., if he contracts muscles in the lower limbs or elsewhere at the same time as he clenches his fist) promotes further rise in blood pressure.

Under the conditions of the experiment, it is evident that emotional factors are reduced to a minimum or absent altogether. At the very least it seems safe to say that no more emotion is present when the subject clenches his fist or stiffens his legs than when he tries to relax. Evidently, therefore, emotional variations do not account for the variations in pressure which correspond with variations in skeletal muscle contraction. The indications are that progressive tension in a muscle group tends to elevate systolic and diastolic pressures, and that this tendency increases if contraction spreads to other groups. Accordingly, the present findings lend further support to the view that in individuals with normal, as well as with increased, pressure (essential hypertension), the pressure levels at any instant vary to an important and determinable extent with the magnitudes of contraction in the various skeletal muscles.

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THE TREATMENT OF EDEMA BY RECTAL ADMINISTRATION OF DIURETICS*

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THE intramuscular and intravenous administration of mercurial diuretics has become widely accepted as a reliable treatment for the edema of congestive heart failure. It seems generally agreed that undesirable systemic or local reactions or subjective symptoms occur infrequently and are seldom of sufficient severity to contraindicate their use. In recent years mercurial diuretics have been applied in the form of suppositories, with the view of rendering their administration more convenient. It is the purpose of this report to review the literature on mercurial diuretic suppositories to date and to evaluate the present status of such preparations for the treatment of edema. Results of additional clinical studies will be included.

Apparently the first report on this method of administration of mercurials was published by Natanson[1] in 1930. In this study, salyrgan, which had been used parenterally for several years, was applied rectally in a glycerin base. In 1934 Engel[2] reported on the use of mercurin† in a cocoa butter base. Only these two mercurial diuretics have been available as suppositories up to the present time. Subsequent investigations have been concerned exclusively with the mercurin suppository. In Table II a summary is given of those papers of which the data are reasonably complete.

All observers have found that the mercurin suppository produces a definite diuresis, comparable to that resulting from the intravenous administration of mercurials, although in most cases quantitatively less. Thus, Thomson[3] obtained an average urinary output of 2,360 c.c. on the day following the administration of suppositories, compared with averages of 3,000 and 2,670 c.c. following the intravenous injection of mercupurin and salyrgan, respectively. Analogous data were reported by Fulton[4] and by Herrmann and Dechard.[5] The simultaneous administration of acid salts has been found to enhance greatly the activity of the mercurin suppositories.

Fulton,[4] Parkinson and Thomson,[6] Thomson,[3] and Herrmann and Dechard[5] all observed that the effect of the suppository was largely spent within twenty-four hours, but on the other hand, Peters[7] found that the diuresis occasionally lasted for forty-eight hours.

Rapidity of absorption has been demonstrated by Fulton,[4] Parkinson and Thomson,[6] and Engel[2] who observed active diuresis even in cases where the

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†This preparation, as well as mercupurin, is known in Europe as novurit.

suppositories were retained for only fifteen minutes to two hours. There is, of course, no evidence that absorption is always, or even usually, so rapid. It may be pointed out here that the majority of workers found the diuresis to be greater when a cleansing enema or an aperient preceded the administration of the drug.

Failures to obtain diuresis following the insertion of the suppositories have been comparatively few, although it is possible that all the failures may not have been reported. Thomson[3] obtained no diuresis in 12 of 200 instances in which suppositories were administered to 11 patients. Fulton[4] reported only 1 patient in a series of 25 who failed to respond to mercurin suppositories, although diuresis was obtained in this individual after parenterally administered mercurials. There were also 6 patients in his series who did not respond to either method of administration. It should be noted that, as with parenteral diuretics, patients whose edema was the result of cirrhosis of the liver usually were less responsive than those with edema of cardiac origin.

Engel[8, 9] has shown clinically that the excretion of mercury following the use of suppositories is quite irregular. This has been confirmed experimentally.[10]

In contrast to the marked unanimity of opinion concerning diuretic efficiency, there is considerable disagreement regarding the local toxicity of mercurial suppositories. Kisch,[11] Christian,[12] Kleeberg,[13] Herrmann and Dechard,[5] and Caughey[14] apparently observed neither subjective symptoms nor objective signs. Fulton,[4] Parkinson nad Thomson,[6] Thomson,[3] Engel,[2] and Wuhrmann[15] reported complaints of slight rectal burning, mild diarrhea, or desire for defecation, but considered these insignificant. Flexner[16] found that irritation occurred only in two cases of his series, and attributed this to the presence of hemorrhoids.

On the other hand, Natanson,[1] Dennig and Krause,[17] Budnitz,[18] and Peters[7] have attached more significance to the occasional occurrence of rectal burning, discomfort, tenesmus, and diarrhea. All of these workers have resorted to the addition of anesthetics or other agents to the suppositories in more or less successful attempts to relieve the subjective symptoms. Even Engel,[8] who considered the toxicity of the suppositories slight, found it desirable to use tincture of opium to relieve pain and discomfort. Furthermore, Engel[2] tested a suppository in which theophylline was combined with mercurin, but noted no change in the degree of irritation. Most clinicians agree that inflamed hemorrhoids, anal fissures, or proctitis are contraindications to the use of mercurial suppositories.

All the clinical reports cited concerning the local toxicity of mercurial suppositories have been based, so far as it is possible to determine, on the subjective symptoms of the patients to whom they were administered. Knowing that mercurin and salyrgan may cause local irritation and necrosis when administered intracutaneously or intramuscularly,[19] DeGraff, Cowett, and Batterman,[20] and at about the same time Crawford,[21] performed proctoscopic examinations on patients before and after suppositories were inserted. The results of

TABLE I

SUMMARY OF LITERATURE ON MERCURIN SUPPOSITORIES

OBSERVER	YEAR	NO. OF CASES	TOTAL NO. OF SUPPOSITORIES FOR ALL PATIENTS	COMPARISON OF DIURETIC EFFICIENCY WITH THAT OF THE PARENTERAL MERCURIALS	LOCAL TOXICITY	AUXILIARY MEDICATION
Dennig and Krause	1935	22	ca. 34	Almost the same	In many patients burning, diarrhea, tenesmus	Anesthesin with suppository partially successful in preventing local irritation
Fulton	1936	25	90	Comparable to mercurial diuretics intravenously	Burning in a few patients	
Kisch	1936	3	4	Volume of diuresis about 90 per cent of that from mercupurin	None	
Kleeberg	1936	Not reported	Not reported	Usually equal	None	
Peters	1936	28	Not reported	Slower in action but almost equal in volume	3 patients with burning, diarrhea, tenesmus	Anesthesin used to reduce toxicity
Engel	1937	10	Not reported	Slower in action but usually equal in volume	Not more than a sensation of heat and desire for defecation	Tincture of opium used to relieve pain
Herrmann and Dechard	1937	70	95	Volume of diuresis 80 and 85 per cent of that from mercupurin and salyrgan, respectively (after enema)	None reported	
Parkinson and Thomson	1937	11	208	Volume of diuresis about 80 per cent of that from mercurial diuretics intravenously	4 patients, mild diarrhea 2 patients, slight discomfort 2 patients, considerable discomfort	
Caughey	1938	2	90	Not reported	None	
Flexner	1938	12	20	Equal	Irritation only in cases with previous rectal pathology	

this work, of which a summary is given in the first four rows of Table II, indicated that the subjective symptoms may be accompanied by visible rectal pathology. Out of a combined total of 34 patients who received the salyrgan suppository, 14 (41 per cent) showed rectal irritation, and 5 (15 per cent) definite ulcerations. No ulcerations were observed after the use of the mercurin suppository, and only 4 out of 31 patients (13 per cent) exhibited injection of the mucosa.

TABLE II

COMPARISON OF PROCTOSCOPIC FINDINGS AFTER RECTAL ADMINISTRATION OF MERCURIAL DIURETICS IN MAN

DRUG	NO. PATIENTS	NO. WITH SUBJECTIVE SYMPTOMS, BURNING, ETC.	PROCTOSCOPIC OBSERVATIONS		NO. PATIENTS WITH SYMPTOMS OF ANY KIND
			NO. WITH INJECTED MUCOSA	NO. WITH ULCERATIONS	
Mercurin (Crawford)	16	Not reported	4	0	4
Mercurin (DeGraff)	15	5	0	0	5
Salyrgan (Crawford)	16	Not reported	9	2	9
Salyrgan (DeGraff)	18	10	3	3	10
Modified salyrgan	36	4	2	0	4

From this review of the literature the following conclusions are reached: (1) The mercurin suppository is a useful diuretic. Its effectiveness is less than that of either salyrgan or mercupurin administered parenterally. Its action is enhanced by premedication with ammonium chloride and by previously emptying the bowel by means of an aperient or enema. Insufficient published reports are available to warrant a statement concerning the diuretic efficiency of the salyrgan suppository. (2) Salyrgan is capable of producing distinct lesions in the rectal mucosa as well as pain, diarrhea, and tenesmus. Mercurin, while seldom producing demonstrable changes in the mucosa, occasionally gives rise to the same subjective symptoms. The presence of rectal pathology should be considered a contraindication to the use of these drugs in the form of suppositories.

In an effort to overcome the irritating properties of the salyrgan suppository, a number of modifications were tested experimentally in this laboratory. The results, which have been reported elsewhere,[10] indicated that a preparation composed of an equimolecular mixture of salyrgan sodium theophylline and salyrganic acid theophylline was the most advantageous. This drug, which will be called "modified salyrgan suppository," was found (in contrast to mercurin and salyrgan) to cause practically no rectal irritation in animals and to give rise to a twenty-four-hour urinary excretion of mercury which was equal to that after salyrgan.

Because these favorable results were noted experimentally, it was felt that clinical investigation of its local toxicity was warranted. Further, the diuretic activity of the modified salyrgan and of mercurin suppositories was studied in patients with congestive heart failure.

Clinical Study of the Local Toxicity of the Modified Salyrgan Suppository.—Thirty-six patients were chosen, regardless of clinical diagnosis, the only criterion being that they were in a satisfactory condition to allow proctoscopic

TABLE III

COMPARISON OF MERCURIN SUPPOSITORY (M. S.), MODIFIED SALYRGAN SUPPOSITORY (M. S. S.), MERCUPURIN (M.), AND SALYRGAN THEOPHYLLINE (S. T.) DIURETICS

CASE NO.	DIURETIC	DATE	AMM. CHLORIDE GM.	INITIAL WT.	WT. FIRST DAY	LOSS FIRST DAY	MAX. LOSS	DAYS REQUIRED FOR LOSS
1	M.—2 c.c.	4/ 7	6.0	129½	118	11½	11½	1
	M.—1 c.c.	4/21	6.0	109¼	104½*	5	5	1
	M. S.	5/13	6.0	118	116	2	2	1
	M.—2 c.c.	5/20	6.0	118	113½	4½	4½	1
2	M. S.	5/24	-	181	168½*	12½	12½	1
3	M.—2 c.c.	3/10	-	139	131*	8	9	2
	M. S.	3/23	-	135½	136½	0	0	-
	M.—2 c.c.	3/26	-	138	131½	6½	6½	1
4	M. S.	7/15	-	175	168½	6½	6½	1
	M. S.	7/19	-	170½	165½	5	5	1
	M. S.	7/24	6.0	173	165	8	8	1
	M.—2 c.c.	7/27	6.0	170	159	11	11	1
	M.—2 c.c.	7/30	6.0	163	154	9	9	1
5	M.—2 c.c.	6/19	-	128½	128	0	0	-
	M. S.	6/26	3.0	129	129	0	0	-
	M.—2 c.c.	6/29	6.0	130	126½	3½	3½	1
	M.—2 c.c.	7/27	-	113¾	110¼	5½	6½	2
6	M. S.	5/19	-	111	113½	0	0	-
	M.—2 c.c.	5/23	-	113	109	4	4	1
7	M. S.	5/19	-	118	115	3	11	3
	M. S.	6/ 5	-	113	107½	5½	8½	2
	M. S.	6/20	Theocin, 2 Gm. per day	111	101*	10	14½	2
8	M.—2 c.c.	4/28	6.0	211½	204¼	7¼	8	2
	M.—2 c.c.	5/ 4	6.0	200¼	198½	1¾	1¾	1
	M.—2 c.c.	5/ 9	6.0	194¾	190½	4¼	5¾	2
	M. S.	5/20	6.0	186½	185½	1	1	1
9	M.—2 c.c.	5/17	-	194½	188	6½	6½	1
	M. S.	5/23	-	193	177	16	16	1
	M.—2 c.c.	6/ 5	-	182	175	7	7	1
	M. S.	6/10	-	182	175½	6½	6	2
	M. S.	6/18	-	183	180½	2½	2½	1
	M. S.	6/29	-	181	174½	6½	6½	1
	M. S.	7/ 6	-	186	181	5	5	1
	M.—2 c.c.	7/15	-	194	188	6	6	1
10	M.—2 c.c.	5/ 9	4.0	165½	157	8½	8½	1
	M. S.	5/26	6.0	160	131½	8½	8½	1
	M.—2 c.c.	6/ 2	6.0	157½	145½*	12	12	1
	M.—2 c.c.	6/ 8	6.0	149½	145½	4	4	1
11	M. S. S.	9/19	6.0	149¼	148	1¼	1¼	1
	M.—2 c.c.	9/28	6.0	148	139*	9	10	2
12	M. S. S.	9/ 5	-	146	145½	½	½	1
	M.—2 c.c.	9/ 8	-	146½	142	4½	8½	2
	M. S. S.	9/14	3.0	138	132*	6	6½	2
13	M. S. S.	11/26	-	204	200	4	4	1
	M.—2 c.c.	11/29	-	199	185½	13½	14½	2

*Weight at which patient was edema free.

TABLE III—CONT'D

CASE NO.	DIURETIC	DATE	AMM. CHLORIDE GM.	INITIAL WT.	WT. FIRST DAY	LOSS FIRST DAY	MAX. LOSS	DAYS REQUIRED FOR LOSS
14	M. S. S.	9/30	6.0	111½	110	1½	4½	2
	M.—2 c.c.	10/12	6.0	107	94	13	?	?
	M.—2 c.c.	10/13	6.0	94	90	4	4	1
15	M.—2 c.c.	8/ 5	3.0	143	132	11	11	1
	M. S. S.	8/17	3.0	138	135	3	3	1
	M.—2 c.c.	8/22	3.0	139½	131½	8	8	1
	M. S. S.	8/29	3.0	139½	138½	1	3½	2
	M.—2 c.c.	9/ 2	3.0	137½	131*	6½	6½	1
	M. S.	9/13	3.0	139	138	1	2	2
16	M. S. S.	12/14	3.0	134	130	4	8	2
	M.—2 c.c.	12/17	3.0	123	113	10	10	1
17	M.—2 c.c.	12/13	3.0	163½	156½	7	8	2
	M. S. S.	12/19	3.0	157½	157	½	½	1
	M. S. S.	12/21	3.0	158½	160	-	0	-
	S. T.—2 c.c.	12/22	3.0	160	156	4	4	1
	M. S. S.	12/26	3.0	158	154	4	6	2
18	M.—2 c.c.	12/12	3.0	128	125	3	3	1
	M.—2 c.c.	12/14	3.0	128	124	4	4	1
	M.—2 c.c.	12/17	3.0	127½	122½	5	5	1
	M.—2 c.c.	1/11	3.0	145	137	8	8	1
	M. S. S.	1/16	3.0	141½	143	-	0	-
	M. S. S.	1/18	3.0	145	143	2	2	1
	S. T.—2 c.c.	1/21	3.0	145½	140½	4	4	1
	M. S. S.	1/23	3.0	142	142½	-	0	1
	M.—2 c.c.	1/24	3.0	142½	134½	8	10½	2
	M. S.	1/28	3.0	139	139½	-	0	1
	M.—2 c.c.	1/29	3.0	139½	131	8½	8½	1
	M. S. S.	2/ 3	3.0	137½	137	½	½	1
	S. T.—2 c.c.	2/ 4	3.0	137	130½	6½	7½	2
	M.—2 c.c.	2/ 8	3.0	134	130½	3½	3½	1
19	M. S. S.	3/ 1	3.0	163	157¾	5¼	6	2
	M. S. S.	3/ 8	3.0	160	156½	3½	5½	2
20	M. S. S.	3/22	6.0	185½	184	1½	1½	1
	M. S.	3/27	6.0	185	178	7	7	1
	M. S. S.	3/31	6.0	181	177	4	4	1
	M. S.	4/ 4	6.0	178½	172	6½	6½	1
21	M. S. S.	3/31	3.0	147¾	146	1¾	1¾	1

TABLE IV

CASE NO.	DIURETIC	DATE	AMM. CHLORIDE GM.	AVERAGE URINARY OUTPUT IN C.C. BEFORE DIURETIC	URINARY OUTPUT IN C.C. AFTER DIURETIC	DAYS REQUIRED FOR LOSS
22	M. S. S.	11/17	3.0	958	2,683	1
	M. S. S.	11/20	3.0	1,283	2,775	1
	M. S. S.	11/23	3.0	1,256	2,425	2
	M.—2 c.c.	11/26	3.0	965	3,540	1
	M. S. S.	11/27	3.0	686	1,920	1
	S. T.—2 c.c.	12/ 2	3.0	611	2,160	1
	M. S.	12/ 6	3.0	794	1,010	-
	S. T.—2 c.c.	12/ 9	3.0	660	2,160	1
	M.—2 c.c.	12/13	3.0	868	2,330	1
	M.—2 c.c.	12/16	3.0	795	1,900	1
	M.—2 c.c.	12/21	3.0	745	2,950	1

examination. A history with reference to previous rectal disease was carefully recorded, and the presence of hemorrhoids, fissures, and other lesions was noted. Proctoscopic examinations* were made shortly after a cleansing enema and before the suppositories were inserted. The patients were instructed to avoid defecation, if possible, for twenty-four hours. Proctoscopic examinations were repeated in each case twenty-four hours after the administration of the mercurial. Routine urine examinations for albumin and formed elements were in every instance essentially negative.

Of the 36 patients studied, 4 complained of a burning sensation following the insertion of the suppository. Upon proctoscopy, one of this group of 4, and one other who had had no subjective symptoms, exhibited definite injection of the rectal mucosa. No instances of ulceration were observed. Examination before administration of the drug revealed internal hemorrhoids in each of these 5 patients. The results, however, cannot be assigned entirely to the initial pathology, for 12 other cases with hemorrhoids, 2 of which were ulcerated and one case with anal fissure, were entirely free from symptoms, and the rectal mucosa appeared unchanged. While it is not advisable to use any mercurial suppository in the presence of ulcerated hemorrhoids and anal fissures, the absence of toxicity in the foregoing cases emphasized the low degree of irritating properties of the modified salyrgan suppository.

The results are summarized in the last row of Table II and are thus compared with those obtained under similar condition of study for salyrgan and mercurin. It is evident that the modified salyrgan suppository is distinctly superior to the original salyrgan suppository, and is at least no more irritating than the mercurin suppository.

Clinical Study of Diuretic Potency of Modified Salyrgan Mercurin Suppositories.—The diuretic effectiveness of mercurin and modified salyrgan suppositories has been investigated in 22 patients with congestive heart failure. The mercurin suppository contains 200 mg. of mercury, and the modified salyrgan suppository,† 158 mg. of mercury. The suppositories were administered only after the patients had had a preliminary period of bed rest and the weight had become constant. In those receiving digitalis, the diuretic was not given until complete digitalization was obtained and the weight remained constant on a maintenance dosage. In following the diuretic response, it was found to be more accurate to follow the daily weight curve, as this fluctuated far less than the urinary output. It also was a good guide in ascertaining the amount of fluid available in the body for diuresis. It has previously been emphasized[22] that the range between the weight before and after diuresis, and the level obtained when the patient is edema-free, is the best available indicator for the effectiveness of a diuretic. Patients were weighed at the same time each day on a scale accurate to 2 ounces. The daily fluid intake was limited to 1,200 c.c., and the salt intake was approximately 5 Gm. Ammonium chloride was given to 16 patients in doses of 3 to 6 Gm. daily.

*We are indebted to Dr. Max P. Cowett, proctologist to the Third Medical Division of Bellevue Hospital, for his interest and kindness in supervising many of these examinations.

†The mercurin suppository was the same as that in general use and was supplied by Campbell Products, Inc. The modified salyrgan suppository was supplied by the Winthrop Chemical Co. under the research number ST 3815.

Three hours after a cleansing enema, the suppository was inserted and the patient was advised to retain it as long as possible. In none of the cases was the suppository expelled in less than six hours, and in most of them it was retained almost twenty-four hours.

The protocols are presented in Tables III and IV. The mercurin suppository was administered 23 times to 14 patients, and the modified salyrgan suppository was given 24 times to 12 patients, 4 patients receiving both preparations. Seventeen patients also received mercurial diuretics intravenously. In 8 of these the suppository was given prior to the parenteral therapy.

A loss of at least 3 pounds in body weight within forty-eight hours of the administration of the diuretic was considered a significant response. In one patient who was too ill to be weighed and for whom the daily urinary output had to be relied upon to determine diuresis, a urinary volume of twice the basal level was considered significant.

A summary of the responses to the trials with the mercurial diuretics is presented in Table V. The percentages of significant diuretic responses with both types of suppository are definitely low compared with that of the parenterally administered diuretic. These results would indicate that while good diuresis may follow the administration of a mercurial diuretic in the form of a suppository, it could be depended upon only in 60 per cent of the cases.

TABLE V

SUMMARY OF TRIALS WITH MERCURIAL DIURETICS SHOWING TOTAL SIGNIFICANT RESPONSES

Salyrgan theophylline was employed intravenously 5 times during the study, but because of the small number of cases, tabulation of the results is omitted.

DIURETIC	TRIALS	DIURETIC RESPONSE	FAILURES	PER CENT SUCCESSES
Mercurin suppository	23	14	9	60.8
Modified salyrgan suppository	24	14	10	58.3
Mercupurin, intravenously	41	39	2	95.1

In the majority of cases the diuresis was complete within twenty-four hours. However, in eight instances following administration of either suppository, a further loss of 2 pounds or more within the next twenty-four hours, and in one instance a loss of 8 pounds during the next forty-eight hours, were noted. Following intravenous administration, there were two instances of significant weight loss during the second twenty-four-hour period.

One patient responded to suppositories with significant diuresis after ammonium chloride therapy had been started, whereas earlier trials had been unsuccessful. While we are in agreement with others that acid salts enhance the action of mercurial diuretics, several failures to respond to mercurial suppositories occurred during the administration of ammonium chloride.

Evidences of Renal Irritation.—Two patients showed changes in the urine during these investigations. Case 4, a 74-year-old man with arteriosclerotic and hypertensive heart disease, showed increasing albuminuria after the third mercurin suppository and, following the first intravenous injection of mercupurin three days later, developed marked albuminuria, innumerable hyaline

and granular casts, and white blood cells. The patient died suddenly three weeks later. At autopsy, the kidneys were normal, grossly, but microscopically the collecting tubules exhibited an occasional calcium deposit in their walls. Case 5, a 38-year-old man with active rheumatic heart disease, showed similar urinary changes following an intravenous injection of mercupurin, given three days after a mercurin suppository had proved ineffective. Cessation of the use of all mercurial diuretics resulted in a return to normal within three weeks, after which time mercupurin was repeated with resulting good diuresis and absence of evidences of renal irritation.

DISCUSSION

The mercurin and modified salyrgan suppositories have been shown to produce minimal demonstrable changes in the rectal mucosa, although they may at times produce local discomfort and burning.

The mercurial suppository is an efficient diuretic, but it can be depended upon only 60 per cent of the times it is administered. The results with the modified salyrgan suppository are less striking than those produced by the mercurin suppository, but it must be remembered that the former contains 158 mg. of mercury compared to 200 mg. in the latter.

The problem arises as to what further therapy would be advisable should the suppository fail to promote diuresis where this is imperative for the welfare of the patient. If diuresis does not occur, it is interesting to speculate that possibly that mercury absorbed from the rectum continues to accumulate in the kidney in amounts that normally would have been excreted. Therefore, if a parenteral diuretic is subsequently given, additional mercury reaches the kidney and may be sufficient to produce tubular damage. The two patients presenting evidence of renal irritation, when an intravenous mercurial was given three days after the suppository, may represent examples of such an occurrence. Therefore, it is to be emphasized that, if the diuresis is not satisfactory after the rectal administration of a mercurial diuretic, the parenteral route should be resorted to only after an appreciable time interval has elapsed to allow the body to care adequately for the previously absorbed mercury.

SUMMARY

1. The mercurin and modified salyrgan suppositories produce only minimal changes in the rectal mucosa, but they may occasionally cause local discomfort and burning.

2. Mercurial diuretic suppositories may produce a definite and clinically effective diuresis, but their dependability is only 60 per cent.

3. The possible danger of administering a mercurial diuretic intravenously soon after a suppository is discussed.

We are very grateful to Dr. Arthur C. DeGraff under whose guidance these investigations were conducted.

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THE EXCRETION OF SQUASH SEED GLOBULIN AND BLOOD PROTEINS IN THE URINE AFTER INTRAVENOUS INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN*

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THE fact that the parenteral injection of foreign proteins into animals causes proteinuria has been recognized for some time. Since Berzelius' observation of this phenomenon, a number of studies of the effect of the injection of foreign protein have been reported in the literature. Inasmuch as the literature on this subject has been recently reviewed by Briggs,[1] only those papers more specifically related to the present discussion will be cited here. In summary, it may be stated that most of these previous investigations have been carried out with complex mixtures of antigenic proteins, such as egg white and blood sera. The excretion of proteins was tested for, in almost all cases, by means of chemical tests which are not sufficiently delicate to detect very small traces of protein, and are not, except in rare instances,† capable of identifying the protein. It seemed desirable, therefore, to study the elimination of a pure protein and to investigate at the same time the excretion of blood proteins following the injection of the foreign protein. Two such studies have been carried out in this laboratory, one using crystallized ovalbumin, the other using crystallized squash seed globulin for injection. The two studies were carried out for the most part simultaneously and, as far as possible, by the use of similar techniques. The excretion of proteins in the urine of injected dogs was followed by testing for the injected protein and for dog serum albumin and pseudoglobulin by means of the precipitin reaction.

The results of the investigation using crystallized ovalbumin have already been reported by Briggs.[1] The results of experiments with crystallized squash seed globulin are reported in this paper. This protein had not previously been used in this type of study. Two somewhat similar proteins, edestin and excelsin, were used by Mendel and Rockwood,[3] who reported that they were not excreted in unchanged form. The tests used by these investigators, however, were not capable of detecting very small traces of protein.

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†In some cases the protein used for injection allowed more specific deductions to be made. Thus Pavy,[2a] after the injection of isinglass, detected both gelatin and coagulable protein in the urine. Parisot and his co-workers[2b] were able to show the excretion of globin and blood proteins following the injection of globin.

PREPARATION OF MATERIAL

The proteins used were prepared in as pure a form as possible. Crystallized squash seed globulin was prepared according to Osborne's method,[4] and purified by four recrystallizations. The blood proteins were prepared by Welker's method.[5] All antisera for these proteins were obtained by intramuscular injection of rabbits with the individual proteins adsorbed on aluminum hydroxide, according to the method of Hektoen and Welker.[6] Each antiserum had a titer of at least 1:100,000, and was specific for the antigen used in its preparation.

PRECIPITIN TESTS

All precipitin tests were made by the contact layer method. A drop of the solution to be tested in progressive dilutions was layered over a drop of antiserum, and the highest dilution of the substance which would give a white band precipitate at the point of contact after standing for one hour at room temperature was determined.

IMMEDIATE EFFECT OF A FOREIGN PROTEIN ON THE KIDNEY

In order to determine the immediate effect of a foreign protein on the kidney of the dog, three medium-sized dogs were injected with crystallized globulin, and urine specimens were collected for an hour. This was accomplished by inserting a No. 8 hard rubber catheter into the bladder of a male dog, and after a preliminary period of ten to twenty minutes, introducing 10 c.c. of a 1 per cent solution of crystallized squash seed globulin in 6 to 7 per cent sodium chloride solution into the lesser saphenous vein. The urine excreted over short time intervals was collected and tested by the precipitin reaction for the presence of crystallized squash seed globulin, dog serum pseudoglobulin, and dog serum albumin. In all three experiments the urine before the injection of the foreign protein gave negative tests for both dog blood proteins. The results of these experiments are summarized in Table I.

TABLE I

TIME OF INITIAL APPEARANCE OF PROTEINS IN THE URINE FOLLOWING THE INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN

DOG	WEIGHT IN KG.	MINUTES AFTER INJECTION OF SQUASH SEED GLOBULIN BEFORE APPEARANCE OF		
		CRYSTALLIZED SQUASH SEED GLOBULIN	SERUM PSEUDOGLOBULIN	SERUM ALBUMIN
31	8.2	7	7	14
32	6.4	10	10	15
33	6.8	5	5	5

Effect of Repeated Injections of a Pure Foreign Protein on the Elimination of the Foreign Protein and the Blood Proteins.—Male dogs, weighing 6 to 14 kg., were used as experimental animals. During the period of injection the dogs were kept in metabolism cages on a complete diet, and the urine was collected under toluene. Each series of injections was preceded by a control period varying from ten to eighty days. During the control period the animals were

kept under experimental conditions, and occasional samples of urine were collected and examined for the presence of dog serum pseudoglobulin and dog serum albumin.

One per cent solutions of crystallized squash seed globulin dissolved in 6 to 7 per cent sodium chloride solution were injected into the leg veins. The injections were repeated within two to six days, so that the physiologic action would take place in an immune, rather than a sensitive, animal. After the completion of the series of injections, the dogs were kept in stock pens, or cages, and returned to the metabolism cages occasionally for periods of observation. The urine specimens were filtered and tested by the precipitin reaction for the presence of crystallized squash seed globulin, dog serum pseudoglobulin, and dog serum albumin.

Ten adult male dogs and one female puppy were given 4 to 35 injections of crystallized squash seed globulin. The duration of the injection period was determined by the effect of the solution injected on the veins of the dog's legs. In some animals the solution caused rapid occlusion of the vessels; in others this action appeared more slowly. The animals may be divided into the following groups:

Group 1.—Dogs 1, 2, 3, 5, and 10 received from 64 to 152 c.c. of 1 per cent squash seed globulin in from 6 to 13 doses, extending over periods of from thirteen to ninety-eight days. Dog 4 received 528 c.c. of this foreign protein solution in 35 injections during a period of one hundred and five days.

Group 2.—Dogs 7, 8, 9, and 11 received from 10 to 72 c.c. of the foreign protein solution in from 10 to 20 doses, extending over periods of from fifty-seven to one hundred days.

Group 3.—Dog 6 received 202 c.c. of 1 per cent squash seed globulin solution in 4 injections over a period of ten days.

TABLE II

REPEATED INJECTION OF CRYSTALLIZED SQUASH SEED GLOBULIN

DOG	WEIGHT IN KG.	NO. OF INJECTIONS	AMOUNT INJECTED IN GM.	DURATION OF INJECTION PERIOD IN DAYS	DURATION OF OBSERVATION PERIOD AFTER LAST INJECTION IN DAYS
1	11.0	8	0.79	24	1,555
2	7.7	6	1.52	21	2
3	11.3	7	1.44	13	7
4	11.3	35	5.28	105	33
5	9.1	6	0.64	14	7
10	10.0	13	1.05	98	52
7	13.6	10	0.38	57	336
8	10.9	20	0.72	113	
9	5.6	5	0.10	21	Died
11	9.1	11	0.51	85	57
6	9.3	4	2.02	10	Died

Table II shows the amount of squash seed globulin injected into each animal, the duration of the injection period, and the length of the period of observation following the last injection.

EXCRETION OF SQUASH SEED GLOBULIN

In the case of 5 of the 6 dogs of group 1, analysis of the urine excreted during the injection period showed that about 35 per cent of the urine samples contained squash seed globulin. In the case of Dog 10, however, only 3 of 57 specimens gave a positive test for the protein injected. With the dogs of group 2, which received smaller doses of foreign protein, a smaller fraction (about 15 per cent) of the urine excreted during the injection period gave positive reactions for the protein injected. One dog of this group, Dog 11, showed little tendency to excrete squash seed globulin, which appeared in but 3 of 48 samples. These results are summarized in Table III.

TABLE III

EXCRETION OF SQUASH SEED GLOBULIN

DOG	NUMBER OF TESTS MADE*	POSITIVE TESTS WITH DIFFERENT DILUTIONS OF URINE					TOTAL NUMBER OF POSITIVE TESTS	PERCENTAGE OF TESTS POSITIVE
		UNDILUTED	1:10	1:100	1:1,000	1:10,000		
1	54	10	4	4	1	2	21	39
2	26	6	2	1	0	0	9	35
3	17	4	0	1	0	0	5	29
4	104	36	1	1	0	0	38	35
5	18	7	0	0	0	0	7	39
10	57	0	0	1	0	2	3	5
7	53	9	0	0	0	0	9	17
8	97	12	2	0	0	0	14	14
9	18	3	1	0	0	0	4	22
11	48	0	0	0	0	3	3	6
6	8	3	2	0	0	0	5	63

*These figures include urine excreted for seven days following the last injection, except in the following cases: Dogs 6 and 9 died following the last injection, and only one urine sample was obtained after the final injection. Dog 2 died two days after the final injection.

In the animals of group 1 the excretion of squash seed globulin decreases after the first 3 or 4 injections. This tendency is shown in Table IV. Opie[7]

TABLE IV

PER CENT OF URINE SPECIMENS GIVING POSITIVE REACTIONS FOR SQUASH SEED GLOBULIN AT DIFFERENT STAGES OF THE INJECTION PERIOD

DOG	DURING THE FIRST PART OF INJECTION PERIOD	DURING THE LAST PART OF INJECTION PERIOD
1	50	27
3	44	13
4	55	27
5	55	22

has reported similar findings with ovalbumin. He expressed the belief that as immunization proceeds the injected animal acquires an increased ability to get rid of the injected protein. Some of Briggs' findings[1] are in accord with Opie's opinion.

After the final injection of crystallized squash seed globulin, the elimination of this protein practically ceases after two to four days. Occasional samples, however, show the presence of the foreign protein for a period of eight to twenty-one days after the final injection. Dog 1 was observed, one

TABLE V

EXCRETION OF BLOOD PROTEINS

DOG	BEFORE INJECTION			AFTER INJECTION						
	NO. OF TESTS MADE	POSITIVE TESTS		NO. OF TESTS MADE	POSITIVE TESTS		NO. OF POSITIVE TESTS IN HIGHER DILUTIONS			PER CENT OF POSITIVE TESTS IN HIGHER DILUTIONS
		NO.	%		NO.	%	1:10	1:100	1:1,000	
Pseudoglobulin										
1	5	0	0	131	124	95	69	5	0	56
2	6	3	50	25	25	100	15	0	0	60
3	5	5	100	17	17	100	9	2	0	65
4	6	1	17	140	103	74	8	1	0	6
5	6	3	50	19	15	79	0	0	0	0
10	20	15	80	77	69	90	18	0	0	23
7	26	9	35	58	53	91	10	0	0	17
8	54	17	32	102	88	85	9	0	0	9
9	13	10	77	18	8	44	1	2	0	17
11	14	5	36	74	58	78	11	0	0	15
6	15	14	93	8	7	88	3	1	0	50
Albumin										
1	5	0	0	131	124	95	41	2	1	34
2	6	2	33	25	25	100	17	0	0	67
3	5	5	100	17	17	100	10	2	0	71
4	6	4	67	140	131	94	16	1	0	12
5	6	6	100	19	19	100	1	2	0	5
10	20	20	100	77	73	95	18	0	0	26
7	26	5	19	58	32	55	6	0	0	10
8	54	15	28	102	63	62	5	2	0	7
9	13	5	38	18	8	44	3	1	0	22
11	14	5	36	74	69	93	16	0	0	22
6	15	15	100	8	8	100	5	0	1	75

year after the final injection, to resume the excretion of small traces of squash seed globulin in several urine samples over a period of twenty-three days (see Table VI).

The amount of squash seed globulin excreted represents only a small part of that injected. The major portion must be either stored in the tissues, metabolized in the body, or excreted in a form which does not react with the specific antiserum. Such an immunologically altered form might result from the combination of the foreign protein with blood proteins. Welker, Andrews, and Thomas[8] have suggested that albuminuria is a result of a protective mechanism in which toxic substances are combined with blood proteins with the formation of a foreign protein which is excreted. It was not possible in this investigation to show that crystallized squash seed globulin formed such combinations.

Hunter[9] has expressed the opinion that the nonexcreted fraction of parenterally injected foreign proteins is digested by proteolytic enzymes elaborated by leucocytes. This hypothesis is based on his observation that during the immunization of rabbits with ox serum and ox serum proteins, each injection was followed by a leucocytosis which increased with each injection.

EXCRETION OF BLOOD PROTEINS

Before injection the urine of most of the dogs gave occasional tests for one or both of the blood proteins. Such positive tests were obtained only on

the undiluted urine, never, except with Dog 6, with urine that had been diluted with 9 or more parts of saline. Immediately after the injection of squash seed globulin, the excretion of the blood proteins was increased. This increased excretion can be noted in the greatly increased frequency with which the urine gives positive tests for these proteins, and by the fact that a number of samples after injection give positive tests when diluted with 9 parts of saline; occasional samples give positive tests when diluted with 99 or more parts of saline. The increase in the excretion of blood proteins was observed in all the dogs tested. The findings are listed in Table V. This increase in the excretion of blood proteins continues after the final injection of squash seed globulin has been given and the foreign protein no longer appears in the urine.

The excretion of foreign protein was always accompanied by the elimination of one or both blood proteins. Two different dogs on one occasion excreted hemoglobin as well as the two plasma proteins. These urines did not contain red blood cells nor did they hemolyze dog erythrocytes. Squash seed globulin was present in one of these hemoglobin-containing urines but not in the other.

The excretion of blood proteins continues long after the urine has ceased to contain squash seed globulin. The excretion of these proteins cannot be correlated with any known factor. Samples usually contained both blood proteins in equal amounts; at times the amount of pseudoglobulin was greater than that of albumin, at other times it was less. Some urine specimens contained only one of the serum proteins, and occasional samples were protein free. The ratio in which these proteins were excreted was not related either to the difference in their molecular size nor to the difference in their concentration in the blood. The process, therefore, cannot be considered as one of simple filtration.

Table VI gives the results of the urine analyses of Dog 1. The results are, for the most part, typical of those obtained with the animals of group 1.

The amounts of protein found in these urines were too small to be detected by the usual chemical tests and would not be considered clinically as pathologic. On the occasions when hemoglobin was excreted, the condition was only transitory and cleared up within a few hours. The histologic examination of sections of liver, spleen, and kidney showed no changes which could be attributed to the experimental procedure. These results are similar to those obtained by Briggs[1] in her study with ovalbumin. Brull[10] has found that dogs can excrete egg white for weeks without presenting a subsequent tendency to true proteinuria.

SUMMARY

The injection of crystallized squash seed globulin into the blood of medium-sized dogs was followed by its appearance in the urine within five to ten minutes. It continues to be excreted for a period of twenty-four to seventy-two hours.

Table VI

Excretion of Squash Seed Globulin and Blood Proteins Following Intravenous Injection of Crystallized Squash Seed Globulin (Dog 1)

Time after initial injection in hours	Amount of 1% cryst. squash seed globulin injected in c.c.	Precipitin reactions of urine with antiserum for		
		cryst. squash seed globulin	serum pseudo-globulin	serum albumin
Control		0	0	0
	5			
5		4	2	2
22		5	1	1
24		3	1	1
45		1	1	1
72		0	1	1
96		0	0	1
124		0	1	1
147		0	1	0
171	5			
173		0	2	2
189		2	2	1
193		5	3	1
216		2	2	0
241		0	2	1
262		1	2	2
286		0	0	1
288	10			
291		1	1	1
294		3	3	1
309		2	1	2
314		0	3	1
334		3	2	1
339		1	2	1
340		0	2	2
362	15	0	2	2
364		1	1	1
381		1	0	1
405		0	2	1
429		0	1	1
436		0	2	1
457	9			
459		0	2	3
460		1	2	2
476		1	3	4
478	17			
480		1	1	1
481		0	2	2
500		0	2	2
523		0	2	1
526	5			
527		0	2	1
548		0	1	2
561		3	2	1
595		0	1	1
598	13			
602		0	1	1
620		0	2	1
626		1	2	2
644		1	2	2
668		0	1	1
692		0	2	3
706		0	2	1
763		0	2	1
768		0	1	1
812		0	2	1
836		0	2	2
860		0	2	2
884		3	2	2
911		0	2	2
958		0	2	2
1,006		1	1	2

TABLE VI—CONT'D

TIME AFTER INITIAL INJECTION IN DAYS	AMOUNT OF 1% CRYST. SQUASH SEED GLOBULIN INJECTED IN C.C.	PRECIPITIN REACTIONS OF URINE WITH ANTISERUM FOR		
		CRYST. SQUASH SEED GLOBULIN	SERUM PSEUDO-GLOBULIN	SERUM ALBUMIN
43		0	1	2
45		2	0	1
46		0	0	0
49		0	0	0
50		0	0	1
51		0	1	2
52		0	1	2
53		0	1	1
220		0	2	2
221		0	1	1
222		0	1	1
385		0	1	1
388		0	1	2
390		0	2	1
391		2	2	2
391		1	2	1
394		0	2	1
395		0	2	2
396		0	2	2
397		0	2	1
398		1	1	1
399		0	2	1
400		0	0	0
401		0	2	1
403		0	1	1
404		0	2	1
405		1	2	1
407		1	2	1
408		1	2	2
409		0	2	2
410		1	2	1
412		1	2	1
414		1	1	1
415		0	2	1
416		0	1	1
425		0	2	1
426		0	2	1
428		0	1	1
429		0	1	1
431		0	1	1
432		0	2	1
434		0	1	1
435		1	1	1
436		0	1	1
437		0	1	1
438		0	1	1
774		0	2	1
775		0	1	1
776		0	2	1
781		0	1	1
782		0	2	2
785		0	2	2
1,172		0	2	2
1,173		0	2	1
1,174		0	2	2
1,574		0	2	2
1,575		0	2	2
1,576		0	1	1
1,577		0	3	0
1,578		0	2	2

0 = No reaction.
1 = Reaction in undiluted urine.
2 = Reaction in all specimens through the 1:10 dilution.
3 = Reaction in all specimens through the 1:100 dilution.
4 = Reaction in all specimens through the 1:1,000 dilution.
5 = Reaction in all specimens through the 1:10,000 dilution.

Following repeated injections, the amount of squash seed globulin excreted tends to decrease. Some foreign protein is excreted at irregular intervals after the last injection.

The amount of squash seed globulin excreted represents only a small portion of that injected. The larger portion could not be accounted for.

The appearance of squash seed globulin in the urine was always accompanied by either serum albumin or serum pseudoglobulin, or both. Repeated injection of squash seed globulin did not cause an increase in the amount of serum proteins excreted.

The injection of crystallized squash seed globulin into medium-sized dogs caused neither immediate kidney damage nor had a cumulative effect on the kidney.

I wish to express my appreciation to Dr. William H. Welker for his advice in the conduct of this investigation and to Dr. George Milles for his kindness and cooperation in examining the histologic material.

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THE FASTING EXERCISE BLOOD SUGAR CURVE: A GUIDE FOR THERAPY IN DIABETES MELLITUS*

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INCREASING experience with protamine zinc insulin has brought into sharp relief a difference in its effectiveness in persons with mild and severe diabetes. The degree of severity seems to be a basic factor in this form of therapy, and it appears that by measuring it, a guide for the efficient use of protamine zinc insulin might be established.

Efforts toward determining the type of diabetes were made long before the introduction of the longer lasting insulins. Petrèn[1] early observed that in some diabetic persons the blood sugar curve tended to drop toward the afternoon, irrespective of meals. Schmidt[2] classified diabetic persons as insulin sensitive if they were asthenic, and as insulin insensitive if they were sthenic. Himsworth[3] and MacBryde[4] developed insulin sensitivity tests designed to differentiate good from poor responses to standard insulin. Klatskin,[5] however, has recently pointed out that the division of diabetic persons into relatively insulin sensitive and resistant groups is an artificial one, while Joslin feels that it is not so simple to classify persons with diabetes.

Of particular interest is a practical method proposed by Seyderhelm and Oesterich[6] for predetermining the need for standard insulin. Diabetic patients were given a small carbohydrate meal the evening before, after the first blood specimen the following morning, and again at noon. Blood specimens were taken while fasting in the morning, and before the noon and evening meals, and the values were plotted as the pattern for the particular patient. Those showing a distinct reduction in the noon value were considered benign and did not require insulin. Where the curve followed an ascending course, the diabetes was severe and required insulin.

More recently, Greene[7] has conducted fasting blood sugar studies which offer a rational basis for grading the severity of diabetes and determining the potential response to protamine zinc insulin. Three types of blood sugar patterns were observed: (1) those in which all readings were within normal limits; (2) those in which the first reading was above normal, with a drop in subsequent determinations on continued fasting; and (3) those in which the first reading was above normal, with a rise in subsequent determinations on prolonged starvation. In group 2 it was evident that endogenous insulin was available, while in group 3 it was lacking. The former, persons with mild diabetes, would do well on Allen's starvation regime, while the latter, persons with more severe diabetes, would require insulin.

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From these observations of Greene and the studies of Seyderhelm and Oesterich, it was believed that a method might by devised whereby the severity of diabetes could be gauged and the response to treatment with protamine zinc insulin could be predicted. Since Greene's classification of diabetic persons studied during starvation appears fully as adequate as that of Seyderhelm and Oesterich, whose patients were given small carbohydrate meals, it was decided to develop patterns during starvation. However, a shortening of the time allotted for the study of each patient seemed desirable both from the practical standpoint and for the protection of the severe diabetic person from serious acidosis. Observation soon disclosed that patients could comfortably tolerate a six-hour period of study without undue risk and with no added harm if they were allowed to exercise or to continue at their regular work. The addition of this latter factor, exercise, served to emphasize the distinction between the mild and the severe forms of the disease, since it has been shown by Marble and Smith[8] that exercise increases the consumption of sugar in normal persons and in patients with mild diabetes, and decreases it in persons with severe diabetes.

TABLE I

NO.	PATIENT	AGE	BLOOD SUGAR			THERAPY
			INITIAL	AT 3 HR.	AT 6 HR.	
1	J. B. C.	60	280	190	184	Diet
2	M. S.	56	142	111	86	Diet
3	L. B.	41	80	71	57	Diet
4	Mrs. M. B.	51	125	115	87	Diet
5	N. G.	47	133	115	106	Diet
6	Mrs. P. D.	66	143	121	120	Diet
7	Mrs. W. S.	59	80	77	76	Diet
8	O. M.	61	100	108	100	Diet
9	Mrs. B. T.	48	180	151	133	Diet
10	Mrs. E. E.	59	220	160	166	Diet
11	Mrs. L. Z.	57	210	186	133	Diet
12	Mrs. M. Z.	49	170	142	133	Diet
13	Mrs. R. B.	50	144	125	95	Diet
14	Mrs. M. P.	66	166	153	129	Diet
15	V. A.	49	105	105	87	Diet

TABLE II

NO.	PATIENT	AGE	BLOOD SUGAR			THERAPY
			INITIAL	AT 3 HR.	AT 6 HR.	
49	J. M.	30	192	152	133	25 PZI 10 Standard
50	Mrs. S. G.	57	88	69	69	20 PZI

As for the usefulness of the procedure in predicting the response to protamine zinc insulin, it was felt that those patients, who by the character of their response possessed an available supply of endogenous insulin, would be adequately controlled by diet with or without a single daily dose of protamine zinc insulin. Those who showed a lack of endogenous insulin would not be controlled in this simple manner.

When put into use, the method consisted in having the fasting patient appear in the morning for three blood specimens to be taken at three-hour

intervals. Studies on 50 diabetic persons were carried out. The results, together with the type of therapy that controlled the diabetes adequately, are presented in the accompanying tables. The fifteen patients in Table I range in age from 41 to 66 years. The highest initial blood sugar level is 280 and the lowest is 80 mg. per cent. All show a descending level from the initial determination, indicating an ability to produce endogenous insulin; hence a mild degree of diabetes controllable by diet alone.

Patients 3, 7, 8, and 15 show figures altogether within normal limits. These patients had been on restricted diets for varying periods, and the diabetes was controlled before the tests were performed. Here the results are similar to those in normal persons and indicate only adequacy of control.

An estimate of the severity of the disease can obviously not be made when the test is performed on patients under treatment. This is further borne out in Table II. Patient 49 was being transferred from regular insulin to protamine zinc insulin, and at the time of the test was receiving 25 units protamine zinc and 10 units standard insulin. While the curve descends from a high initial level, indicating a mild grade of diabetes controllable by protamine zinc insulin alone, it has obviously been influenced by the administered insulin and fails to give a true picture. This patient has actually required the combined use of standard and protamine zinc insulin for control. The second patient, receiving 20 units protamine zinc insulin daily, shows figures within the normal limits, the curve simulating that of the normal person and indicating satisfactory control by diet combined with one dose of protamine zinc insulin.

Table III includes 19 patients showing patterns in which the curve descends from an initial level higher in most instances than those in Table I. All these patients were properly controlled by diet combined with one daily injection of protamine zinc insulin. An exception is noted in the case of patient 30, whose three-hour level is higher than the initial determination. This patient was in the hospital recovering from acute pyelitis. It is probable that the infection distorted the curve, giving it the appearance of that found in a person with severe diabetes. Patients 19, 20, and 27 present flat curves, with none of the values much higher than the normal. These patients have a mild form of diabetes controllable by diet alone. A single daily dose of protamine zinc insulin is being given to keep them aglycosuric since they refuse to stay within dietary bounds. Here the studies, having established the mildness of the disease and its potential control by simple measures, serve as an aid in detecting dietary disobedience.

In Table IV are 12 patients ranging in age from 20 to 82—5 being below age 40. Patients 35, 36, 37, and 38 show a uniformly ascending type of curve. Patients 39 and 40 show the first two figures of equal value and the third lower. Patient 41 shows the last two figures higher than the first. Patients 42, 43, and 44 show the middle figure highest. Patients 45 and 46 show the middle figure lower than the others. None of these patients could be controlled by diet alone or combined with a single dose of protamine zinc insulin.

TABLE III

NO.	PATIENT	AGE	BLOOD SUGAR			THERAPY
			INITIAL	AT 3 HR.	AT 6 HR.	
16	E. S.	52	330	166	143	10 PZI
17	Mrs. S. N.	49	390	280	235	25 PZI
18	V. J.	47	250	224	186	10 PZI
19	L. A.	47	130	100	92	10 PZI
20	L. S.	55	133	130	130	15 PZI
21	Mrs. A. W.	77	186	140	100	15 PZI
22	A. R.	73	250	228	200	10 PZI
23	Mrs. E. B.	73	305	280	280	10 PZI
24	Mrs. M. R.	66	266	228	190	30 PZI
25	Mrs. R. L.	66	225	218	180	10 PZI
26	S. K.	59	200	172	150	20 PZI
27	S. R.	59	133	133	133	10 PZI
28	S. D.	46	500	400	235	30 PZI
29	A. P.	32	500	450	400	40 PZI
30	Mrs. R. R.	59	345	400	330	15 PZI
31	Mrs. L. W.	60	360	270	220	40 PZI
32	Mrs. E. B.	52	210	180	133	20 PZI
33	G. B.	14	270	228	220	30 PZI
34	J. B.	59	133	122	111	10 PZI

TABLE IV

NO.	PATIENT	AGE	BLOOD SUGAR			THERAPY
			INITIAL	AT 3 HR.	AT 6 HR.	
35	Mrs. R. L. B.	20	520	600	620	25 PZI 20 Standard
36	F. L.	36	360	450	500	40 PZI 10 Standard
37	Mrs. K. S.	49	250	290	330	25 PZI 20 Standard
38	Mrs. J. R.	58	138	160	200	15 PZI 10 Standard
39	R. H.	66	380	380	166	25 PZI 10 Standard
40	R. K.	37	200	200	160	20 Cryst.* in A.M. 10 Cryst. in P.M.
41	C. C.	34	214	266	266	30 Cryst. in A.M. 20 Cryst. in P.M.
42	Mrs. E. F.	50	136	166	154	20 PZI 10 Standard
43	Mrs. E. A.	40	275	350	255	45 PZI 20 Standard
44	W. S.	70	250	280	210	20 PZI 10 Standard
45	Mrs. M. S.	59	200	166	210	20 PZI 10 Standard
46	Mrs. B.	82	400	260	400	10 Standard t.i.d.

*Zinc insulin crystals.

TABLE V

NO.	PATIENT	AGE	BLOOD SUGAR			THERAPY
			INITIAL	AT 3 HR.	AT 6 HR.	
47	Mrs. H. S.	39	152	150	140	Diet
48	Mrs. E. W.	35	100	99	100	Diet

All required in addition a varying amount of regular insulin. Patients 40 and 41 had mild hyperthyroidism. In both, better control was maintained through the use of two daily doses of zinc insulin crystals.

It is evident that despite the variety in patterns presented by this group not a single one resembled the flat curve of the normal person or the descending curve of the person with mild diabetes. All simulated to some extent the ascending curve of the person with severe diabetes.

Two patients with symptoms of acromegaly and diabetes of pituitary origin are presented in Table V. Both show a flat type of curve resembling the normal, patient 47 being at a higher level. This patient is successfully controlled by diet alone. The second patient originally required insulin for adequate control, but within the past year has required neither insulin nor a severely restricted diet. The test was made early in the remission of the diabetes and is certainly normal in appearance. No conclusion of note can be drawn from these data—excepting that the figures verify the observed mildness of diabetes associated with acromegaly.

SUMMARY AND CONCLUSIONS

A knowledge of the degree of severity in diabetes is important for predicting the manner of response to therapy. The person with mild diabetes with a fair supply of endogenous insulin is readily controlled by diet alone or combined with a single daily dose of protamine zinc insulin. The person with severe diabetes, with little or no endogenous insulin, is not so easily controlled, requiring regular insulin in addition to the protamine zinc insulin.

A test for gauging the severity of diabetes has been described here. This consists in making three blood sugar estimations at three-hour intervals on the fasting patient while he is ambulatory. The resulting curves fall into three groups: (1) a continuous descent from the initial reading, all levels being not far above the normal values; (2) a continuous descent from the initial reading, with all levels considerably higher than the normal values; and (3) a group of curves, none of which show a continuous descent, with at least one of the last two readings being higher than the initial value. In the first two groups are the persons with mild diabetes that are readily controlled by diet alone or combined with a single dose of protamine zinc insulin. In the third group are the persons with severe diabetes controlled only with more than one dose of insulin.

Of 50 persons with diabetes studied, 15 fell into the first group, 19 into the second, and 12 into the third. Two patients with acromegaly and diabetes showed flat curves, indicating a mild degree of diabetes since they simulated the response of the normal nondiabetic person. The remaining two patients were tested during treatment with insulin. One, receiving both regular and protamine zinc insulin, showed a descending curve typical of mild diabetes, when, in fact, his was a severe diabetes requiring both types of insulin for control. The other, receiving a single daily dose of protamine zinc insulin, showed a flat type of curve resembling the normal. Four similar curves were noted in the group of patients with mild diabetes on restricted diets for vary-

ing periods before testing. This experience indicates that the test is not applicable to patients under treatment—since in these it offers only a measure of the adequacy of treatment.

The test appears to be most useful in the new or untreated patient with diabetes. By gauging the severity of his disease, a greater degree of efficiency, with economy of time and expense, may be attained in planning therapy.

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OBSERVATIONS ON A SULFANILAMIDE SOLUTION

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A SOLUTION of sulfanilamide for oral administration, as profound in its action as other forms of sulfanilamide and with no greater tendency to produce side actions, is desirable because of the ease with which it may be administered, especially to children, and the accuracy with which dosage can be measured. Such a solution has been prepared and subjected to a series of experimental tests to determine its clinical applicability. The solution* is essentially a mixture containing in each fluidounce approximately 30 grains of sulfanilamide and 4 fluid drams of systemic alkalinizer (approximately 29.7 per cent sodium lactate and 10.6 per cent potassium citrate by weight) and glucose.

Three separate series of experiments were conducted, the first two employing the entire solution, and the third a solution made up without potassium citrate but otherwise identical with that used in the first series.

METHOD OF STUDY

In order to determine tolerability, toxic dosage, and minimum lethal dose, 27 guinea pigs, averaging 230 Gm. in weight, were employed. Increasing quantities of the sulfanilamide solution were administered by stomach tube, in divided doses, twice a day for periods of at least twenty-one days.

*The solution of sulfanilamide, known as sulfanilamide with citra-lactate, was furnished through the courtesy of Donley-Evans & Co., St. Louis.

A second study was conducted with 7 rabbits, employing the sulfanilamide solution, sulfanilamide powder, and prontosil, all in sulfanilamide equivalent dosage, in order to evaluate them comparatively. The drugs were all administered in divided doses at the same time daily. The blood concentration of free sulfanilamide was determined one, four, eight, and ten hours after administration, using the method of Marshall.[1] The residual blood concentration of free sulfanilamide in milligrams per cent was determined eighteen to twenty-four hours after administration of the various preparations used.

Microscopic sections of the brain, liver, stomach, and kidneys of the guinea pigs and rabbits, which had been given lethal doses of the sulfanilamide solution, were examined for evidence of pathologic changes in these organs.

In the third series of experiments sulfanilamide solution without potassium citrate was employed. Six guinea pigs, averaging 251 Gm. in weight, were each given 12 c.c. of the solution (representing approximately 12 grains of sulfanilamide) per day in three divided doses, or approximately 3.2 Gm. per kilogram per day, for a period of forty days. Another group of 6 guinea pigs, averaging 247 Gm. in weight, received 15 c.c. each of the sulfanilamide solution daily in three divided doses, or approximately 4.0 Gm. per kilogram per day. These experiments were conducted to determine the tolerance dose of the sulfanilamide solution without potassium citrate.

RESULTS

In the first series of experiments it was found that the guinea pigs tolerated 14.8 c.c. of the sulfanilamide solution (approximately 1 Gm. of sulfanilamide) per kilogram of body weight per day for periods of at least twenty-one days, without manifesting symptoms of toxicity or loss in weight. The solution was found to be no more toxic than sulfanilamide powder in equivalent dosage.

When given in doses of 17.3 c.c. (approximately 1.17 Gm. of sulfanilamide) per kilogram of body weight per day, the sulfanilamide solution produced very mild toxic symptoms, but the animals survived this dose for periods of at least twenty-one days. Toxic symptoms with 21.7 c.c. (approximately 1.47 Gm. of sulfanilamide) of the solution per kilogram of body weight per day did not occur when the potassium citrate was removed from the solution. The lethal dose, as determined by this series of experiments, was approximately 26 c.c. per kilogram of body weight (approximately 1.76 Gm. of sulfanilamide). Halpern[2] states that the guinea pig is about as susceptible to the toxic effects of sulfanilamide as is the rabbit. Halpern and Mayer[3] state that the toxic dose for the rabbit is 2 Gm. per kilogram. Raiziss[4] and co-workers find that 2 Gm. administered orally is lethal for rabbits. The findings of one of us (F.L.), in a comparison made at the same time, was that the lethal dose of sulfanilamide in guinea pigs was approximately 1.56 Gm. per kilogram per day.

Chart 1 demonstrates the time of appearance of free sulfanilamide in the blood when administered in the form of solution, powder, and prontosil, in sulfanilamide equivalent doses. It may be observed that free sulfanilamide

appears in measurable quantities in the blood of rabbits somewhat more quickly when given in the form of the solution than when administered in other forms; also, that the solution leads to an appreciably higher concentration of sulfanilamide in the blood than does sulfanilamide powder or prontosil.

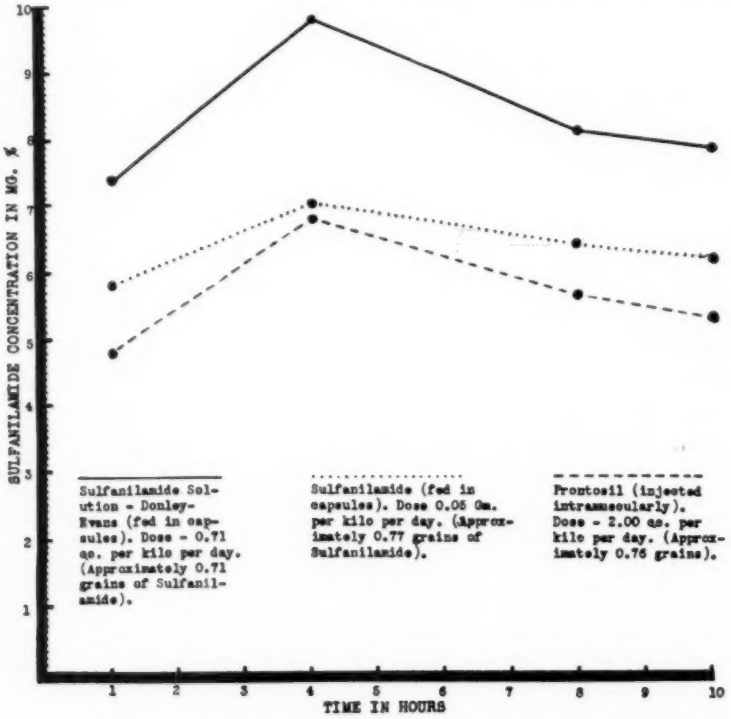
SULFANILAMIDE CONCENTRATION IN MG. %
TIME IN HOURS
Sulfanilamide Solution - Donley-Evans (fed in capsules). Dose - 0.71 gm. per kilo per day. (Approximately 0.71 grains of Sulfanilamide).
Sulfanilamide (fed in capsules). Dose 0.05 Gm. per kilo per day. (Approximately 0.77 grains of Sulfanilamide).
Prontosil (injected intramuscularly). Dose - 2.00 gm. per kilo per day. (Approximately 0.76 grains).

Chart 1.—Blood concentration tests of three forms of sulfanilamide in rabbits.

Chart 2 shows the rate of elimination of sulfanilamide from the blood when administered in various forms. The rate of elimination is no more rapid when the drug is given as the solution than when given in other forms. Since the blood concentration with the solution is somewhat higher than with the powder or prontosil, and since the rate of elimination from the blood is no more rapid with the solution, the residual blood concentration after withdrawal of the drug remains somewhat higher with the solution than with the other two drugs. This fact may indicate that the extent and duration of its action are increased when sulfanilamide is administered in the form of the solution.

The microscopic sections of the brain, liver, stomach, and kidneys of the guinea pigs and rabbits given lethal doses of the sulfanilamide solution revealed no evidence of disease in these organs. There were no significant pathologic changes either in the organs of animals dying within a few days after the administration of large toxic doses or in the organs of animals given mildly toxic doses for periods up to twenty-one days.

In the third series of experiments, the series employing sulfanilamide solution without the potassium citrate, animals given 12 c.c. of the solution per

day in three divided doses made a continuous gain in weight and showed no toxic symptoms. When the dose was increased to 15 c.c., two of the animals died within the first three days; the other four survived until the experiment was concluded at the end of thirty days. Although these animals did not gain weight, they retained their original weight fairly well. They developed mild toxic effects, such as drowsiness and weakness in the hind legs, with some stiffness.

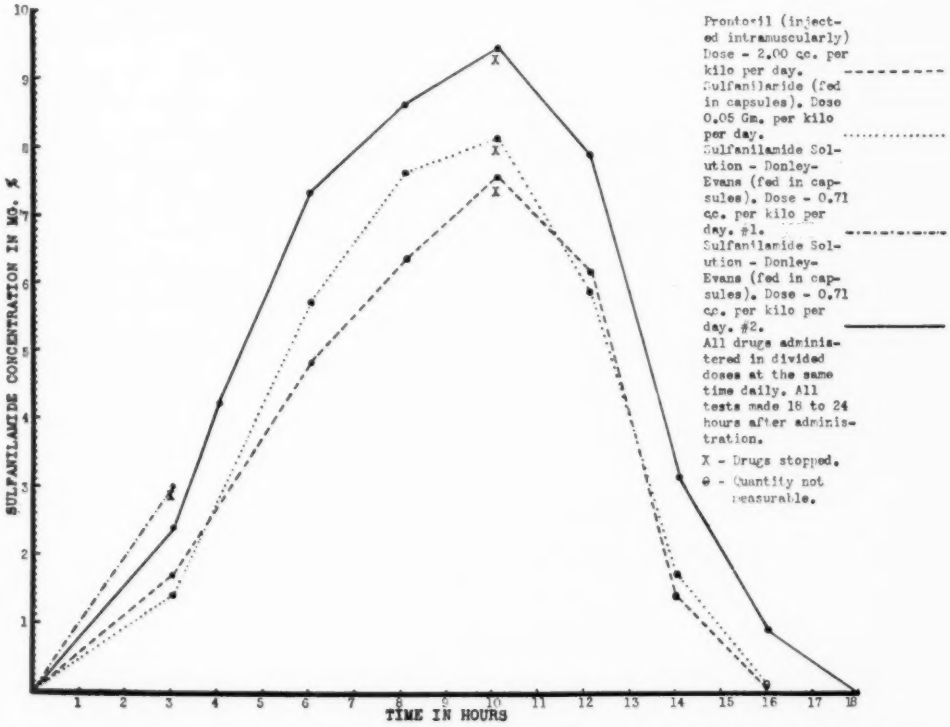
SULFANILAMIDE CONCENTRATION IN MG. %
TIME IN HOURS
Prontosil (injected intramuscularly) Dose - 2.00 cc. per kilo per day.
Sulfanilamide (fed in capsules). Dose 0.05 Gm. per kilo per day.
Sulfanilamide Solution - Donley-Evans (fed in capsules). Dose - 0.71 cc. per kilo per day. #1.
Sulfanilamide Solution - Donley-Evans (fed in capsules). Dose - 0.71 cc. per kilo per day. #2.
All drugs administered in divided doses at the same time daily. All tests made 18 to 24 hours after administration.
X - Drugs stopped.
Θ - Quantity not measurable.

Chart 2.—Residual blood concentrations 18 to 24 hours after administration in milligram per cent.

It appears, therefore, that the sulfanilamide equivalent tolerance dose of the sulfanilamide solution, without potassium citrate, is slightly above 3.2 Gm. per kilogram of body weight per day when given in three divided doses by stomach tube. The administration of 10 c.c. of the solution containing potassium citrate to a 250 Gm. guinea pig apparently exceeds the tolerance dose of potassium citrate for guinea pigs. Hence, it seems that in the guinea pig, symptoms of intolerance of sulfanilamide solution containing potassium citrate are probably due, at least in part, to the large amount of potassium citrate present.

Clinical experience with sulfanilamide solution has confirmed the experimental evidence demonstrating its safety, and the extent and duration of its action. Thus far, after extensive clinical trial, no alarming toxic reactions have been observed. It is our impression that the side actions are definitely more numerous with sulfanilamide in the form of the dry drug than with the solution, when equal doses of the drug are administered.

CONCLUSIONS

1. In the guinea pig and rabbit higher blood concentrations of free sulfanilamide are obtained when the drug is given as the solution than when it is administered in equivalent doses in other forms.

2. The rate of elimination of the sulfanilamide is no more rapid when given as the solution than when given in other forms.

3. The sulfanilamide appears in the blood more rapidly when given as the solution than when administered in other forms.

4. Sulfanilamide solution, therefore, may be expected to be as effective in the treatment of certain infections as any other form of sulfanilamide.

5. From the experimental evidence presented, it can be concluded that there are probably fewer toxic side actions with the sulfanilamide solution than with sulfanilamide in the form of the dry drug when administered in sulfanilamide equivalent dosage.

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THEOPHYLLINE WITH ISOPROPANOLAMINE IN HEART DISEASE*

With Especial Reference to Congestive Failure

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THE treatment of congestive heart failure remains a challenge to the cardiologist's therapeutic ingenuity. Venesection has proved by experience to be a lifesaving measure in congestive failure, but it is too seldom used in our modern techniques. This procedure lessens the peripheral cardiac load and enables more efficient myocardial function. The lessening of pressure in the venous circulation is temporary but frequently of sufficient duration to enable the weakened right heart to regain compensation. A drug capable of lowering venous and spinal fluid pressures, as well as producing capillary dilation, would considerably enhance the value of venipuncture. Our investigations suggest the fulfillment of these criteria in theophylline with isopropanolamine. This is a water-soluble theophylline compound, and represents a combination of theophylline with isopropanolamine.[1] The dilation of the capillaries of the nail bed, as well as lowering of the venous and spinal

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fluid pressures produced by this drug, extends over a period of fifteen minutes, and precludes its use for any but emergency measures as far as the intravenous route is concerned. Intramuscular administration may prove to have a more sustained effect, as will be investigated at a later date. Our observations showed considerable relief from dyspnea and orthopnea shortly after injection, lasting from three to four hours. The clinical improvement was far more sustained than the laboratory studies indicate.

We suggest the use of this drug in congestive heart failure to enhance the effects of venesection. Eyster[2] attributed the value of venesection in congestive heart failure to the reduction of a high venous blood volume. His contention was relief of abnormal initial strain in the right ventricle. Harrison[3] made the observation that cisternal pressure was higher in the recumbent than in the erect position. He thus concluded that a patient with congestive orthopnea secured better pulmonary ventilation in the erect than in the recumbent position. He also found that spinal drainage, in most cases, was followed by a decrease in dyspnea. One of us (H. F. R.)[4] has shown that combined venesection and spinal drainage is followed by even greater relief than is secured from lumbar puncture alone. It is believed that the use of this drug, which attains much the same results as the above-mentioned procedures, will prove of some value in combination with blood letting and lumbar puncture when indicated in cardiac congestion.

METHODS

All readings were taken in the morning or midafternoon, with the patient in the erect position. The persons studied comprised various types of congestive heart disease, including rheumatic, arteriosclerotic, syphilitic, and hypertensive. They were observed with reference to venous, spinal fluid, and arterial pressures, as well as to caliber of the capillaries in the nail fold of the finger. The readings were made at intervals of five minutes up to fifteen minutes directly before and after the intravenous administration of theophylline with isopropanolamine. Normal values for spinal fluid pressure, taken between the third and fourth lumbar vertebrae in the sitting position, we consider as varying from 150 to 500 mm. of water. Normal values for venous pressure at the level of the right auricle, taken in the upright position, we take as from 90 to 120 mm. of water. The spinal fluid pressure was read with an Ayer water manometer. The venous pressure was read with an L glass tube of 7 mm. diameter inserted into the antecubital vein precisely at the level of the right auricle. The capillary diameters in the finger nail fold were determined with a filar micrometer and hair pointer, as described by Duryee and Wright.[5]

OBSERVATIONS

Our series comprised 21 patients. Five had spinal fluid and venous pressure studies as preliminary tests. Nine were observed with regard to spinal fluid, venous pressures, and capillary diameter. The capillary measurements in this series were made by comparison with a hair pointer. A filar micrometer was then used for this purpose in the final group of 7 patients which also included the venous, arterial, and spinal fluid determinations.

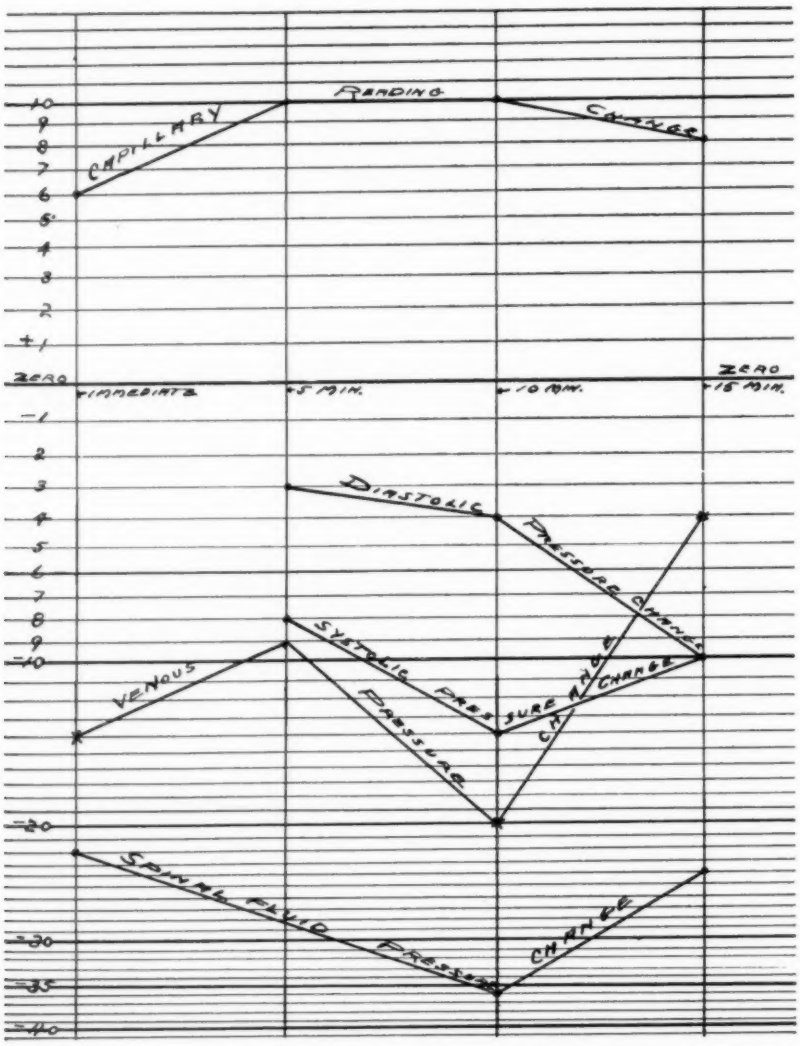

Fig. 1.—Arith-log study of average changes of pressures and capillary readings of micrometer after the intravenous administration of 10 c.c. of 0.025 per cent theophylline with isopropanolamine.

Table I shows a tabulation of the average changes, with the probable error at the four observed points for all the experiments. This indicates a fall of all the pressures with a commensurate dilatation of the capillaries in the fingernail fold. The greatest change occurred ten minutes following the injection. These results were plotted on arith-log paper (Fig. 1), utilizing the suggestion of Pearl[6] that variables showing a rate of change must be coordinated to a base to establish the respective relationship. It is to be noted that the rate of change of venous and spinal fluid pressures from immediately after administration to the ten-minute period are almost parallel. The rate of capillary dilatation rises rapidly, simultaneous with the fall in spinal fluid and venous pressures during the first five minutes. It then remains constant and gradually returns to normal. The diastolic pressure falls gradually

TABLE I

TABULATION OF AVERAGE CHANGES AND THEIR PROBABLE ERRORS

The average readings of 21 patients studied after the intravenous administration of 10 c.c. of 0.025 per cent theophylline with isopropanolamine.

	IMMEDIATE		5 MIN.		10 MIN.		15 MIN.	
	AVG.	PROBABLE ERROR	AVG.	PROBABLE ERROR	AVG.	PROBABLE ERROR	AVG.	PROBABLE ERROR
Spinal fluid pressure	-22	±5.0	-28	±5.0	-36	±5.5	-24	±7.0
Venous pressure	-14	±6.5	- 9	±4.5	-20	±9.0	- 4	±5.0
Systolic arterial pressure			- 8	±3.0	-14	±4.0	-10	±0.6
Diastolic arterial pressure			- 3	±1.0	- 4	±3.0	-10	±0.6
Capillary reading	+ 6	±2.0	+10	±1.0	+10	±2.0	+ 8	±1.0

at first, finally diminishing considerably as the capillary dilatation persists. The end of the ten-minute period marks the greatest lowering of systolic pressure, which then rises with the elevation of venous and spinal fluid pressures.

These observations are in accord with current physiologic concepts. It is well established that spinal fluid pressure fluctuates in direct proportion to venous pressure, and variations of diastolic pressure are similarly closely related to the tone of the peripheral capillary bed.

SUMMARY

The effect of theophylline with isopropanolamine was studied in a series of 21 patients, with reference to capillary dilatation, arterial, venous, and spinal fluid pressures. These pressures showed a definite fall associated with a capillary dilatation. This response to the drug, although comparatively brief, may prove useful in the treatment of congestive heart failure in conjunction with venesection and other appropriate measures. Further study may establish a more sustained effect with the oral or intramuscular administration of this preparation. Our observations showed considerable relief from dyspnea and orthopnea shortly after injection, lasting from three to four hours. The clinical improvement was far more sustained than the laboratory studies indicate.

We wish to express our gratitude to the National Drug Co. for the material supplied for the research problem.

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HYPERGLOBULINEMIA IN GRANULOMA INGUINALE

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GRANULOMA inguinale and lymphogranuloma inguinale, the so-called fourth and fifth venereal diseases, are quite distinct clinical entities in spite of the similarity of their names. The former is essentially a disease of the skin and corium[1] and seldom invades the deeper tissues. Beginning as a small macule on penis or vulva, it spreads as a superficial destructive fungating mass over the pudendum, perineum, and inguinal regions. Scrapings from the mass are characterized by the pathognomonic Donovan bodies. The infection usually responds satisfactorily to tartar emetic injections or, better still, to Fuadin therapy.

Lymphogranuloma inguinale, on the other hand, is essentially a disease of the lymphatic tissues. Beginning as a small macule on the pudendum, it develops deep-seated buboes with areas of induration and softening that later discharge through multiple fistulas. Involvement of the rectum with stricture is characteristic, and the Frei test appears to be pathognomonic. There is no satisfactory specific therapy.

Lymphogranuloma inguinale is almost certainly a virus infection.[2] The nature of the Donovan bodies which have been demonstrated to be the cause of granuloma inguinale[3] is still a matter of dispute, although there is much to be said in favor of Donovan's original contention that they are protozoan in character.

In 1936 Gutman and his co-workers reported a series of serum protein determinations in lymphogranuloma inguinale, their reports, however, being only incidental to more extensive studies. In their first communication,[4] they discussed 12 cases of lymphogranuloma inguinale, all with a positive Frei test. Serum globulin values, ranging from 2.2 to 7.8 per cent, were found; in all but 2 cases the values were higher than 4.1 per cent. Half of the cases had a serum globulin content of 5.0 per cent or higher. In a second series[5] they report 13 cases, all in Negro women with positive Frei reactions; 12 of the 13 were under treatment for rectal stricture. The serum globulin findings in this series were essentially like those in the first; euglobulin determinations ranged from 0.7 to 4.0 per cent, being generally, but not constantly, proportionate to the total globulin values. In a third communication,[6] they reported 35 cases of lymphogranuloma inguinale, all with positive Frei test. The total serum globulin ranged from 2.2 to 8.3 per cent, being 5.0 per cent or higher in nearly half the cases, and above 4.0 per cent in three quarters. The euglobulin ranged from

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0.6 to 4.0 per cent, again being generally, but not always, proportionate to the total serum globulin. Similar findings were reported by Rosen, Rosenfeld, and Krasnow.[7]

The foregoing findings indicate that hyperglobulinemia is a characteristic feature of lymphogranuloma inguinale and suggest that it might help to distinguish it from granuloma inguinale in those borderline cases in which confusion is possible. It seemed worth while, therefore, to look through our files for cases of granuloma inguinale in which blood protein determinations had been made. Seven such cases were found, 6 of them florid, the seventh nearly healed following tartar emetic injections. In some of these the diagnosis had been confirmed by the presence of Donovan bodies; in all of them the picture was quite characteristic. The findings are shown in Table I.

TABLE I

	TOTAL PROTEINS %	SERUM ALBUMIN %	SERUM GLOBULIN %	EUGLOBULIN %	PSEUDO-GLOBULIN %
G. V. (florid)	9.3	3.9	5.4	1.7	3.7
J. H. (florid)	9.40	3.72	5.68	1.18	4.50
S. W. (florid)	8.5	4.6	3.9	1.9	2.0
G. H. (florid)	10.3	4.9	5.4	1.3	4.1
R. W. (florid)	8.25	3.81	4.44	0.90	3.54
P. R. (florid)	8.1	3.4	4.7	1.7	3.0
P. R. (treated)	7.55	4.57	2.98	0.15	2.83

A much larger series would obviously be required to determine the uniformity with which hyperglobulinemia occurs in granuloma inguinale. The interesting feature is the extremely high euglobulin percentage with a somewhat more moderate increase in the total serum globulin.

SUMMARY

Recent reports in the literature indicate that hyperglobulinemia is present in most cases of lymphogranuloma inguinale.

The same phenomenon occurs to a marked degree and with high euglobulin values in granuloma inguinale.

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THE OCCURRENCE OF GUANIDINE-LIKE SUBSTANCES IN THE BLOOD IN ESSENTIAL EPILEPSY*

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NUMEROUS studies of the concentration of guanidine-like substances in the blood have been carried out in the past ten years by various investigators in both normal and pathologically affected subjects, as well as in animals manifesting tetanic or convulsive symptoms, renal and hepatic involvements. A field, apparently missed by previous investigators, in which increase in the guanidine-like substances in the blood might be expected, is represented by the essential epilepsies—especially when the seizure pattern is that of *grand mal*.

Fifteen institutional patients were studied by the colorimetric method of Pffifner and Myers, as modified by Andes and Myers,[1] except that, instead of the final values being read ocularly, more objective readings were taken from a photoelement photospectrometer, with monochromatic light (485 mμ) placed at our disposal in the Kettering Laboratory. This was deemed desirable because (a) Lambert-Beer's law is valid only for monochromatic light; (b) the color of the blank is relatively great compared to the color developed by the guanidine; (c) finally because the maximum absorption of both occurs in a region of low sensitivity to the human eye.

Basal levels were first determined in each case (the average of five taken during apparently normal periods), then serial determinations made during aura, seizure, and one hour after cessation of convulsion. The findings are presented in Table I. These figures show that in the patients with essential epilepsy studied, guanidine content of the blood rises significantly. Blood guanidine level rose during the aura, reached its highest point during the seizure, then dropped back to basal level.

With the foregoing facts demonstrated it becomes possible to speculate on the place of guanidine in the epileptic pathology. We emphasize, however, that though all the cases studied showed the same tendencies, the total number is still small.

Experiment shows that an increase in blood guanidine level is followed by convulsions. Guanidine has powerful action on the neuromuscular junction. The response of the muscle to single shock nerve stimulation is modified as the guanidine concentration is increased. Instead of being followed by a single response, a tetanus is now produced, sustained at times for minutes.

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TABLE I

GUANIDINE VALUES

(Expressed in Milligrams Per Cent)

PATIENT NO.	AVERAGE BASAL LEVEL	AURA	CONVULSION	POST CONVULSION
1	0.50	0.80	1.60	0.61
2	0.41	0.76	1.31	0.53
3	0.37	0.64	1.17	0.51
4	0.31	0.60	0.76	0.50
5	0.45	0.74	1.47	0.56
6	0.38	0.69	1.21	0.54
7	0.42	0.67	1.14	0.54
8	0.36	0.53	0.93	0.51
9	0.42	0.66	1.26	0.52
10	0.47	0.79	1.50	0.58
11	0.47	0.80	1.57	0.52
12	0.37	0.62	0.92	0.51
13	0.30	0.55	0.80	0.50
14	0.49	0.73	1.32	0.57
15	0.5	0.79	1.54	0.61

Thus with sufficient increase in guanidine concentration, any "normal" stimulus may peripherally be so effective as to cause convulsion. Clinically guanidine rise may come from any of the following sources: increased formation; decreased destruction by liver; decreased elimination by kidney. The shape of the curve would tend to indicate the first.

SUMMARY

1. In the instances of essential epilepsy studied, the basal content of guanidine in the blood was found significantly high.

2. All who presented convulsions of the *grand mal* variety showed a blood guanidine rise during the aura reaching a high point during convulsion.

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LABORATORY METHODS

AN APPARATUS FOR MULTIPLE ESTIMATIONS OF CHOLESTEROL IN OXIDATIVE MICROMETHOD*

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RECENTLY I reviewed modifications and factors in the oxidative technique for the microanalysis of blood lipids.[1] In the present study I wish to describe a special apparatus that has proved useful in the oxidative micromethod for blood cholesterol.

The digitonin precipitate of cholesterol requires filtration, washing, and redissolving in boiling methyl alcohol. An apparatus has been devised for this purpose, similar in principle to that previously described,[1] but containing a number of technical improvements and permitting the simultaneous estimation of up to 12 samples; more could be added by enlarging the apparatus. A photograph of the entire apparatus is shown in Fig. 1, and a close-up of two units in Fig. 2.

The framework consists of a base 2 feet wide, 4 feet long, and 7/8 inches thick, set on two crossboards that act as feet and add support. On the back of this base is set a box section, 14 inches wide and 6 inches high. The top of the box section is made of two halves of equal width, the back half being permanently attached to the box section and the front half attached with removable screws (Fig. 2). At intervals circles are cut out along the center of the top of the box section, half of the circle being in the front of the back half and the other half of the circle being in the back of the front half of the top of the box section. These circular holes are made large enough to accommodate snugly the neck and upper part of 1 liter, side-necked, filtering flasks, which sit upon the base within the box section. The diameter of these holes will depend upon the shape of the flasks and the thickness of the wood used in making the top of the box section: a diameter of 3¼ inches was used in making the apparatus illustrated in Fig. 1. The removable front half of the top of the box section enables these filtering flasks to be removed if broken or for emptying.

An upright board, 3 inches high, is placed 1½ inches forward from the back edge of the top of the box section. Running along the back of this upright board is a half inch iron pipe, interrupted by T-joints at intervals; to these joints are attached short lengths of pipe that extend forward through the upright board. On the front of these extensions is a compressed air tap, connected by thick-walled rubber tubing to the side neck of the filtering flasks. The extreme left end of the pipe (the reader's left when looking at the apparatus) is closed. the right end is brought through the upright, and another compressed air tap is attached. This latter tap connects by means of heavy-walled tubing to a Hyvac

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Fig. 1.—A general view of the cholesterol apparatus.

Fig. 2.—A close-up of two units of the apparatus.

pump placed on a shelf attached to the wall in a convenient place just above the apparatus. The apparatus shown in Fig. 1 was originally designed to function with a water pressure filter pump, and an attachment to such a pump through a trap was included in the apparatus; these are shown to the right in Fig. 1. The water pump, being too light, was discarded in favor of the more satisfactory Hyvac pump.

The stem of the sintered glass filters (4G4, Schott and Jen, Jena) is fitted into a No. 0, one-holed rubber stopper, which in turn is inserted into the top of a 15 ml. centrifuge tube that passes through a rubber stopper in the top of the filtering flasks. The bottom of the centrifuge tube is cut off. When appropriate taps are open, from 1 to 6 cholesterol digitonide precipitates may be filtered at one time along the back row of filters.

After the cholesterol digitonide has been filtered free of its mother liquor and the precipitate washed with acetone and ether in the back row of filters, it is transferred in the sintered glass filter with attached rubber stopper to the front row of steam coils for redissolving. Another batch of cholesterols may then be started filtering. Live steam enters the coils from the left of the apparatus through copper tubing, arranged in 6 coils of sufficient diameter to enclose snugly the sintered glass filters. The copper tubing is continuous from one steam coil to the other, and the whole is attached to the front of the box section at a convenient height to permit the oxidation flasks to be inserted and removed. The extreme right end of the steam line empties into a sink. Underneath, and fitting up into the bottom of each steam coil, is a glass suction head made by a glass blower. The top of the glass suction head contains a flanged opening large enough to receive the No. 0 rubber stoppers of the sintered glass filters. There is a side neck attached through thick-walled rubber tubing to a compressed air tap held against the front of the box section. All these taps are connected by piping along the inside of the front of the box section; this piping ends at the right by coming forward through the front board of the box section and is sealed there with another compressed air tap. This latter tap is connected to the tubing running to the Hyvac pump by thick-walled rubber tubing and a T-tube. Suction may be directed either to the front or to the back rows or to both by opening and closing appropriate taps at the right end of each pipe line. T-tubes and clamps attached in the rubber tubing going to the Hyvac pump act as release valves.

The glass suction head is sufficiently wide at the base (1⅜ inches) to fit over the neck and top of 125 ml. glass-stoppered Erlenmeyer flasks (the oxidation flasks). To make an airtight joint with the oxidation flasks, strips of flat rubber tubing, 1¾ inches wide are doubled over the bottom of the glass suction heads. Each suction head is then attached loosely into pliable metal strip clamps fixed to the front of the box section.

ESTIMATION OF BLOOD CHOLESTEROL

The technique of estimating blood cholesterol is summarized here. A more detailed description and discussion, directions for the preparation of reagents, and modifications necessary for special variations have been given in a previous report.[1]

One milliliter of whole blood, serum, or plasma is run slowly into 25 ml. of alcohol-ether solution, 3:1, both redistilled. The proteins are filtered off, washed several times with the extracting fluid, and finally pressed with a cleaned glass rod. The combined filtrates are boiled down to a volume of about 20 ml. in an Erlenmeyer flask, 0.1 ml. of saturated sodium hydroxide is added, and the flask is placed on a steam bath at a temperature sufficient to evaporate the extract to a volume of 1 ml. in thirty to sixty minutes. One milliliter of 25 per cent sulfuric acid and a few drops of phenol red indicator are added, and the flask is placed on top of the steam bath until patches of condensed water vapor begin to appear on the side of the flask. The aqueous layer is then extracted four to six

times with 5 to 10 ml. portions of petroleum ether. Five milliliters of 0.2 per cent digitonin (Merck) in 50 per cent alcohol are added to the combined petroleum ether extracts, and the mixture is concentrated almost to dryness. Ten milliliters of distilled water are added to the precipitated cholesterol digitonide and brought to a gentle boil. After cooling, 10 ml. of acetone are added, and the mixture is shaken. The cholesterol digitonide is then filtered in the apparatus here described, the precipitate is washed in succession with acetone and ether, and is then redissolved in three successive portions of boiling methyl alcohol, each portion being drawn with suction into a 125 ml. glass stoppered Erlenmeyer flask. The methyl alcohol solutions are then evaporated to dryness without burning on a hot plate, the 125 ml. Erlenmeyer flask is transferred to a steam bath, and the last trace of methyl alcohol vapor is blown off with a gentle stream of air. The cholesterol digitonide is then oxidized with 5 ml. of Nicloux reagent and exactly 3 ml. of 1 N potassium dichromate, and heated in an oven for thirty minutes at 124° C. The flask is removed from the oven, and about 75 ml. of ice-cold distilled water are added. The unreduced potassium dichromate is then titrated with standard 0.1 N sodium thiosulfate. The figure thus obtained is subtracted from that of a blank treated in the same manner, but without the original blood, serum, or plasma. The difference is expressed in terms of exactly 0.1 N potassium dichromate, divided by 10.61 and multiplied by 100 to give the number of milligrams of cholesterol per 100 ml. of blood, serum, or plasma.

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A STANDARDIZED MASTIC TEST FOR SPINAL FLUID*

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SENSITIVITY and *specificity* are the two qualities that characterize the value of a serologic test. But a third quality, *constancy*, is important too. In syphilis the difficulties with regard to constancy have been overcome to a large extent, as far as competent laboratories are concerned. This, however, cannot be said regarding the invaluable Lange test and its modifications, the colloid tests of the spinal fluid.

Many formulas have been worked out to guarantee reagents of a constant quality, but none of them is fully satisfactory. For this reason, the colorful reagent of Lange[1] has been replaced to a certain extent by other colloids. Among these, mastic (Emanuel[2]) and other gums have the first place. The resins offer a great advantage since, in preparing the colloids, the procedure can be based on well-known physical factors which are the foundation of serology in syphilis. Sachs and Rondoni have detected the fundamental fact that the reactivity of the Wassermann antigens depends on the speed with which heart extract and saline are mixed. Sachs, as well as Kafka and Jacobsthal,[3] have stated that the same law is valid for the mastic test. As the antigens for the lipoid-precipitation tests, too, obey the law of Sachs-Rondoni, the similarity between lipoid and colloid tests is especially impressive.

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The speed of mixing the saline with heart extract or water with mastic solution determines not only the sensitivity of such colloids for their use as reagents, but also their faculty to become precipitated by other physical influences, especially by salt solutions. The law of Sachs-Rondoni, as broadened for these different conditions, may be summarized in this way: When a colloidal reagent for syphilis is prepared by mixing an alcoholic solution of heart lipoid, resin, etc., with water (or saline), the opacity of the resulting colloid, and, simultaneously, its reactivity toward serum or spinal fluid, increases in the same degree in which the mixing speed during preparation decreases. Toward salt solutions the behavior is the reverse. The colloids are the clearer and the more easily precipitated by salt solution, the faster the mixing had been done. We have the extremes: (1) Slow mixture—dense colloids—high sensitivity for serum and spinal fluid—low sensitivity for salt solutions. (2) Quick mixture—clear colloids—low sensitivity for serum and spinal fluid—high sensitivity for salt solutions. (The reactivity in the Wassermann reaction is substantially identical with the precipitation in the flocculation tests.)

But in practice the determination of the mixing speed is rather inexact. If we request that the mixing be done drop by drop, and be finished within a certain time, the intervals between the single drops (which influence the result in a high degree) cannot be standardized perfectly. In any case, the results with the different modifications of the mastic reaction were unsatisfactory because of a definite lack in constancy.

In the lipoid flocculation tests, mixing the reagents by dropping has been substituted for some years by a two-step-mixing. This can be adopted for the mastic test.[4]

EXPERIMENT

To a row of tubes containing 1 c.c. of a 1 per cent alcoholic mastic solution, distilled water is added as rapidly as possible, in the amount of 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, respectively. Opacity develops rapidly in all tubes within the first few seconds, then increases slowly. After two minutes no perceptible change is noted, and the tubes show different degrees of opacity. If this state is reached, sufficient water is added quickly to each tube to make 4 c.c. of water. The final effect is read five minutes later.

There is present a series of colloidal solutions, the opacity of which increases from tube to tube to a certain point, and then decreases by degrees. All the colloids contain the same amount of water, alcohol, and mastic. They are all prepared with the same mixing speed, and differ from each other only in the fact that water is added in two portions of different amounts. This influences the final density in a manner similar to that produced by different mixing speeds. The significance of this statement is derived from the fact that the law of Sachs and Rondoni is valid here, too. The densest colloid is the most sensitive to spinal fluid and the most resistant to salt solution. The clearest one has the opposite qualities; it is the least sensitive to spinal fluid and the most sensitive to salt solution.

Instead of the mixing speed, the amount of the first portion of water is made the deciding factor. It is evident that with this method it is easy to prepare a series of colloids with exact gradation, and to repeat the preparation of the selected one in a constant way. The highest density and the highest sensitivity are present in the tube in which 1 c.c. of water has been added in the first step. We may state that the ratio of one part mastic to one part water is an "optimum." This ratio is a characteristic, distinguishing peculiarity of all solved substances which can be changed into a colloid by adding water. It is, for instance, 1:0.5 for the original Meinicke extract. To the left and to the right of this optimum we have tubes with decreasing opalescence; that is to say, if we add either more or less than 1 c.c. of water, the colloids are clearer and less sensitive.

One difference, however, exists between the two sections of the series. If the first addition is less than 1 c.c. of water, the peculiarity of the final colloid is not completely determined by the first step, and the speed of the second addition of water is also important in this respect. While after an addition of 1 c.c. or more, the colloidal fate is definite, and the speed of the second step is without distinct influence.

The proper distribution of the whole amount of water between the two portions secures the desired degree of sensitivity. However, we request more than a constant sensitivity. The colloid shall yield a certain constant opalescence, so that even a slight reaction may be noted clearly. In this regard the concentration of the alcoholic mastic solution is the most important point. Usually from a 10 per cent stock solution a 1 per cent routine solution is made. But mastic is a trade product, the composition of which is not fully constant. A various, sometimes considerable, part is insoluble in alcohol. Thus the concentration of the stock and the routine solutions, and of the final colloid, may be lower than expected.

Another still more important requirement depends on the concentration, namely, the correlation between the peak of the curve and the dilutions of the spinal fluid. If a large number of syphilitic spinal fluids is examined with the same reagent, it is striking how constantly the peaks of the curves coincide with the same dilution of the spinal fluid. There are differences between various methods. With colloidal gold the peak of the curve corresponds to the spinal fluid dilution 1:80. With the method for mastic described here the peak of the curve corresponds to spinal fluid 1:8, which is slightly different from the original method and from the "Normomastixreaktion" (Kafka). In each method the different syphilitic curves differ from each other in depth and width, but only exceptionally in the position of the peak of the curve. Even a slight shifting has its significance, provided that such a shifting is not caused by faulty technique.

It is remarkable that the curve is shifting to the right if the concentration of mastic is lowered. To secure constancy of the results the mastic concentration of the colloid must be kept constant by adjusting the dilution with alcohol to the individual specimens of the resin.

The last point to be considered is the balance between the sensitivity to spinal fluid and to sodium chloride. A colloid test that has the proper degree

of sensitivity to the spinal fluid, but is precipitated by the salt solution, is somewhat inconvenient. The use of a salt solution of lower concentration entails some complications which cannot be described here. A small quantity of alkali, as stated by Cutting,[5] and by Kafka and Goebel,[3] raises the resistance against sodium chloride, but influences only slightly the behavior toward spinal fluid.

After one has arrived at these conclusions, the technique is rather simple, if a laboratory is in a position to use a tested resin. A bit more complicated, but without any difficulty for an experienced worker, is the procedure of testing. The following reagents and glassware are needed: (1) Solution of 10 Gm. mastic in 90 Gm. absolute alcohol. After some days the solution is filtered, and the residue is removed. The stock solution can be kept in the ice box without any change in its composition. (2) Absolute alcohol. (3) Distilled water. (4) Aqueous solution of 0.5 per cent sodium carbonate. (5) Tubes 80 mm. long, 10 mm. wide. (6) Racks with 18 tubes in each row. It is convenient to have racks with two rows, one above the other, in such a way that the tops of the lower row of the tubes do not overlap the bottoms of the upper row. (7) Pipettes of 1, 5 and 10 c.c. (8) Graduate cylinders of 10, 25, and 100 c.c. (9) Beakers of 30 and 100 c.c.

Procedure A (With Tested Resin).—The routine solution of mastic is made by mixing 1 part of the stock solution with as much absolute alcohol as directed (usually 1 : 12 to 14, resulting in an approximately 0.7 per cent solution). Alkalinized water, with a content of 0.005 per cent sodium carbonate, is prepared by adding 0.1 c.c. of the 0.5 per cent sodium carbonate solution to each 10 c.c. water. For the preparation of the colloidal reagent, 1 part of routine solution is mixed with 4 parts of alkalinized water, the mixture being performed in two steps.

Example.—To prepare 50 c.c. colloid, three 100 c.c. beakers are supplied with 10 c.c. routine solution, 10 c.c. alkalinized water, and 30 c.c. alkalinized water, respectively.* Into the mastic solution, 10 c.c. water is poured as fast as possible and mixed thoroughly by rotating the beaker. A delay in the mixture brings the uncontrollable factor of speed into the procedure. After an interval of two minutes, the contents of the third beaker, 30 c.c., are added. A moderate variation in the speed is without distinct influence in this stage. After five minutes the colloid is ready for use. It does not change within a few hours, and changes very little in several days. For the dilution of spinal fluid 0.5 c.c. sodium chloride (0.9 per cent) is poured into each of 9 tubes, 0.5 c.c. spinal fluid is added to the first tube, and diluted in the usual manner. One-half cubic centimeter of the colloid is used for each tube, and the result is read off the next day (at least sixteen hours later).

Procedure B (Testing of Mastic).—Different routine solutions are made by diluting the stock solution with absolute alcohol in different proportions (1 : 10 to 15). From each of these[6] solutions a series of colloids is made by varying the first portion of water in the following manner: To a series of tubes containing 1 c.c. of mastic solution, 1.0, 1.2, 1.4, 1.6 c.c., respectively, of al-

*This is a proportion between the components frequently found to give the desired sensitivity of the colloid.

kalinized water are added and made up to 4 c.c. water after two minutes. After five minutes the colloids are ready. All colloids are tried on the same negative and positive spinal fluids, and the most satisfactory reagent is selected as a basis for the routine preparation.* This description is a sketch of our procedure; it is not really intended that all these 24 colloids be tested. Usually the samples of mastic do not differ so much from each other, and the technique may be simplified. From an average routine solution two colloids are prepared by adding, as a first step, 1 and 1½ parts of alkalinized water. According to the results on two spinal fluids, the further testing can be reduced to a few variations. Only for specimens of unusual peculiarities, or for a new kind of resin, is the more extended testing necessary. With the help of this method, reagents have been worked out from 24 different kinds of resin.[6]

A few technical details may be mentioned here. Some specimens of mastic give hyposensitive colloids with the regular technique. The use of 95 per cent alcohol, instead of absolute alcohol, for the preparation of the routine solution raises the sensitivity. The same effect can be arrived at by subdividing the first portion of water.

It remains to define strictly which colloid has to be selected as the most satisfactory one. A colloidal reagent has to fulfill three requirements: sensitivity, appearance, and curve. These qualities have a certain relationship with each other. The mixing speed (or the amount of the first portion of water) influences density and sensitivity in the same direction, while an increase in the concentration of mastic increases the density but lowers the sensitivity. On the other hand, the resin concentration has an influence on the shape of the curve. Consequently, no factor can be considered by itself alone; it must harmonize with the others.

As for the density, the colloidal reagent should be only slightly opalescent. It should become intensely opaque in reaction, but milky appearance is not desirable as it would diminish the visibility of precipitates. The shape of the curve is correct if its peak, on the average, corresponds with the dilution 1:8 of spinal fluid, i.e., the third tube. We determine this point by testing with several "weak" syphilitic spinal fluids.

To define the correct sensitivity, a theoretical conception must be outlined. Between the lipoid tests (mainly of the serum) and the colloid tests (of the spinal fluid) there is a great resemblance in the colloidal fundaments and (omitting the Wassermann reaction) also in the visible happening. In both types of test the characteristic effect can be brought about also by nonsyphilitic sera and spinal fluids and, with "too sensitive" reagents, even by normal sera and spinal fluids. The assumption has been made, therefore, that the lipoid reactions, too, are of merely colloidal character, with only quantitative difference between the syphilitic and the normal. But the colloidal conception is not sufficient to explain fully the positive results in the lipoid tests. Certain physical conditions, it is true, influence the reactivity of the reagents to syphilitic and nonsyphilitic sera in the same direction and to the same degree. But serology has learned to prepare antigens which have an increased specific sensitivity, without considerable in-

*The selected colloid need not be the most sensitive one which corresponds with the colloidal optimum.

crease in the nonspecific reactivity. Therefore, we must conclude that the syphilitic reactions are qualitatively different from the nonsyphilitic ones, and in a practical sense we may call the lipoid tests "specific." Numerous investigations have shown that they are so in a theoretical sense.* The fundamental requirement lies in the chemical composition, as heart extract is the only source for the reagent. The colloidal state of a syphilitic serum, even if it differs from the normal state, has nothing to do directly with the specific reaction. In contradistinction, the effect of nonsyphilitic serum upon heart extract is in most of the cases nothing more than a reaction between colloids. The lipoid colloid can be disintegrated, for instance, by different protein solutions, and serum, being such a solution, is able to precipitate colloids of different chemical origin. In syphilis a specific reaction between antibodies and antigenic fractions of the heart starts the disintegration of the colloidal system, and the latter can be given a certain stability which is not shattered by the influence of normal serum or serum of moderately increased lability. Only a considerable increase in this direction (which often can be demonstrated by rapid sedimentation rate, a positive Takata-Ara test, etc) imparts a serum with the faculty of precipitating a routine antigen.

The condition can be compared with the preparation of dynamite. A highly explosive material, which principally can be blown up by mechanical effects, is brought to a certain stabilization in which only ignition can promote explosion. Once such an influence has started, the further development depends on the original "explosivity." We may say, therefore, that a sort of "safety zone" is laid between the specific and the nonspecific districts, which can compensate for a considerable oscillation in the colloidal state of an individual serum. Add to this the fact that the increased colloidal reactivity, which may disturb the result, occurs principally in severe diseases (carcinoma, tuberculosis, pneumonia, and other febrile diseases). The clinical evidence alone induces us to be careful in the evaluation of the serologic report in such a case. Furthermore, if sera of this type are used for control, hypersensitivity of an antigen will be detected before false results occur in sera of patients with no severe general disease.

The colloidal reactions are quite different in their demarcation of syphilis from other pathologic changes as well as from the normal condition of the spinal fluid. The profound nonsyphilitic changes are expressed in many instances (meningitis, brain tumor, abscess) by the "meningitis curve," the peak of the curve shifting to the right (Lange[1] and Flesch[8]) curve cannot be mistaken for syphilis. It is the case with less marked changes (encephalitis, arteriosclerosis), the curve of which often cannot be distinguished from a syphilitic one. Further, the contrast between a strong positive colloidal curve and a negative lipoid test, which is a striking feature of nonsyphilitic reactions, is unusual in syphilis, and is almost always met with only after intense treatment. A distinct colloidal curve which is not accompanied by a positive lipoid test is, as a rule, not of syphilitic origin, even if there is no "shifting to the right" (multiple sclerosis, some cases of tumor). Only a weak colloidal curve may often go along with a negative lipoid test also in syphilis (e.g., tabes dorsalis). This

*This has been emphasized by H. Eagle.[7]

is another reason why diseases with only a slight change in the spinal fluid producing a weak curve, have a less distinct demarcation from syphilis than those with more marked spinal fluid changes.

The normal spinal fluid, too, has the faculty of producing a change of the syphilitic type in the colloidal reagent. This can easily be demonstrated by using a reagent with increased reactivity. However, there is one point which distinguishes the fact from similar experiences with the lipoid tests: the mechanism of syphilitic and normal curves is identical, for in both instances the curve is a product of a combined action of albumin and globulin (Lange[1, 9]). An increased effect of globulin alone causes a spinal fluid to produce a curve of the syphilitic type.* This occurs not only if the amount of globulin is augmented by syphilis or by another disease, but also if the globulin amount is normal and the reagent more sensitive. For this reason, no definite demarcation can exist between syphilitic and other pathologic, or between syphilitic and normal, spinal fluids. A syphilitic spinal fluid and a nonsyphilitic one, in both of which the globulin alone is increased to the same level, give the same curve, and a syphilitic curve is as strong as the protein content of the spinal fluid differs from the normal condition. Only an increase in the albumin (e.g., meningitis) produces a curve that differs fundamentally by shifting to the right.[11] But also this curve can, in early cases, resemble a syphilitic curve. And exactly in the same degree in which the sensitivity is raised toward syphilis, it is raised toward other pathologic changes and toward normal spinal fluid. There is no safety zone between syphilitic and nonsyphilitic spinal fluids. The aim of adjusting the mastic cannot be to catch the highest possible amount of syphilis, and the lowest amount of nonsyphilis, but by means of a linear gradation to fix the sensitivity in such a way that all spinal fluids with normal protein content barely give a negative result. This is more feasible since the normal content oscillates in narrow limits only. Any change in the protein condition, regardless of its origin, will show up in the curve. The main use of the colloid tests is not so much to base the diagnosis of syphilis upon them, as to answer the question if and how far in a case of ascertained syphilis the central nervous system is involved. For this purpose we can also evaluate a slight reaction which in itself does not differentiate syphilis from another disease or from a meaningless increase of protein in the spinal fluid (during fever). But one condition must be fulfilled, that is, constancy. We repeat: there is no safety zone, no true specific quality, and the syphilitic and nonsyphilitic changes and the normal condition shade into each other.

The manner in which we, nevertheless, can make diagnostic conclusions with regard to syphilis as well as to other diseases cannot be explained here.

In adjusting the sensitivity of the reagent at a certain level one hint is advisable. Although our technique makes the colloids very constant, a continuous control is necessary. A too high sensitivity will be recognizable in a small number of specimens, for—contrasted with the lipoid reactions in such a case—not only single specimens but all of them will show themselves nonspecific. But what about the reagent that is hyposensitive? We do not always have

*Although the merely quantitative character of the characteristic changes in the proteins is not definitely proved, this theory is supported by numerous investigations, among which the publications by Kafka and Samson are especially important.[3, 10]

at our disposal a spinal fluid with a tested reactivity; moreover, we will not omit the possibility given in the colloid test of having the methods at the same level in all laboratories. By adjusting the colloids in a way that all negative spinal fluids give negative results, we are not protected against hyposensitivity. It is, in my opinion, favorable to have a reagent with which the negative spinal fluid gives just a recognizable curve. The slightest degree of change indicates the standard sensitivity, and gives us an excellent control of the constancy which is the purport of my method.

In the Serologic Department of the General Hospital in Vienna, the technique described was routine for fourteen years, and was performed by me in about 70,000 specimens of spinal fluid. The most important observations were those on numerous patients who had undergone malaria treatment in the Wagner-Jauregg Clinic, Vienna, and in cooperation with Dr. B. Dattner, were kept under control for years. By its considerable constancy the standardized mastic test was able to express slightest clinical changes or to foretell a clinical development which much later became manifest. The technique was successful with resin available in this country.

SUMMARY

Besides sensitivity and specificity, constancy is an indispensable quality of a serologic test. In the technique of the mastic test described here, the gradation of the sensitivity is done by adding water to mastic solution in two steps, whereby the amount of the first addition is decisive for the final colloid. In this way, the sensitivity can easily be varied, and the selected reagent can repeatedly be prepared in a constant manner. Some fundamental details are discussed which add to the desired qualities of a colloidal diagnostic reagent. The technique has been useful in about 70,000 specimens of spinal fluid.

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A SIMPLE DEVELOPING TANK FOR ELECTROCARDIOGRAMS

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TO SIMPLIFY the developing of electrocardiograms for the practitioner who does not do x-ray work, I have devised a compact three compartment tank, which makes the process easy and convenient.

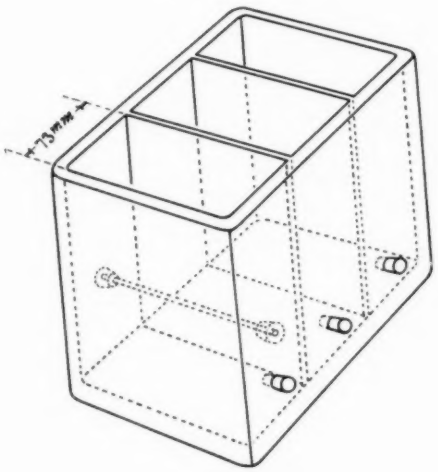

Fig. 1.

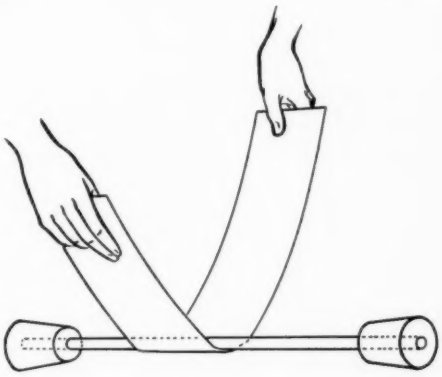

Fig. 2.

An ordinary storage battery, thoroughly cleaned out, presents three excellent chambers, perfectly arranged for the purpose (Fig. 1). The width of each chamber being about 73 mm., any electrocardiographic paper on the market today can be accommodated. The first compartment contains the developing solution. The section is so arranged that the graph may be rolled (emulsion side out) beneath a glass rod, supported at either end by one-holed No. 4 rubber stoppers, as in Fig. 2. The rod is placed about 5 cm. from the bottom, and its location may be adjusted, depending upon the amount of solution in the tank. The rubber stoppers prevent the rod from moving while the paper is rolled to get uniform developing. The middle compartment contains the water, and the third compartment, the fixing solution.

Each compartment possesses a 1 cm. bore hole at the base, about 25 mm. from the bottom, plugged with a No. 00 rubber stopper. This allows for individual emptying and cleaning of a chamber. A cover to fit the tank may be made of wood to keep the solution from outside contamination.

By the use of this tank it is possible to have all solutions within reach at any time. Solutions may be used repeatedly, space is conserved, and uniform development is obtained. It may also be used for ordinary photographic work.

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A COMPARISON OF CEDAR OIL AND OTHER MATERIALS IN THE MAKING OF SLIDES OF ATMOSPHERIC POLLEN*

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FOR the purposes of determining the amount and kind of pollen in the air, Scheppegrell[1] in 1917 exposed plates prepared by applying glycerin to the central square inch of an ordinary microscope slide which had previously been cleaned with alcohol. Various preparations were tried at that time, but glycerin was found to be superior to any other. When the air was extremely moist, the glycerin deliquesced rapidly, but this deliquescence was overcome by substituting boiled linseed oil. In the beginning of his work, Scheppegrell attempted to expose slides at right angles to the direction of the wind; later he found this to be impractical due to the limited range of a single slide. He found the most practical method to be the simple exposure of the slides to the wind without the use of any special apparatus. After the slides had been exposed for twenty-four hours, a drop of Lugol's solution was placed on the glycerin surface, thus facilitating the counting. The grass pollens were stained blue black in contradistinction to the other pollens, which were usually stained brown.

In 1924 Scheppegrell[2] made pollen counts from slides carried in an airplane. These slides were smeared with either glycerin or vaseline. The combination of glycerin and Lugol's solution facilitated the counting, but the preparation was rapidly dried by the great velocity of the wind to which the plates were exposed. This was not the case with the slides on which the vaseline film was employed.

Koessler and Durham[3] exposed slides in various parts of Chicago in 1926. They used corn oil and found it to be more satisfactory than glycerin because it did not absorb moisture and thus distort the pollen grains. They found that stains were unnecessary in their study.

In his study of the atmospheric pollen and botanic flora of the East Shore of San Francisco Bay, Rowe[4] used slides covered with a film of vaseline. The slides were exposed in an upright position, with the vaseline facing the prevailing wind, for a period of twenty-four hours. He found that the pollen grains adhered to the slides covered with white vaseline and retained their dry, shrunken shapes.

Detweiler and Hurst[5] used slides coated with a thin layer of glycerin jelly. The jelly was prepared according to Kaiser's formula and subsequently remelted in a water bath. It was then smeared over the slide with a glass rod, an area of the slide equivalent to the surface of a cover slip being prepared.

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These slides were exposed on window ledges in various parts of Toronto for periods of twenty-four hours. When the slides were collected, a drop of glycerin jelly, protected by a cover slip, was added.

Wodehouse[6] described various stains used in combination with a glycerin jelly. The methyl green stain proved to be the most satisfactory of the staining preparations.

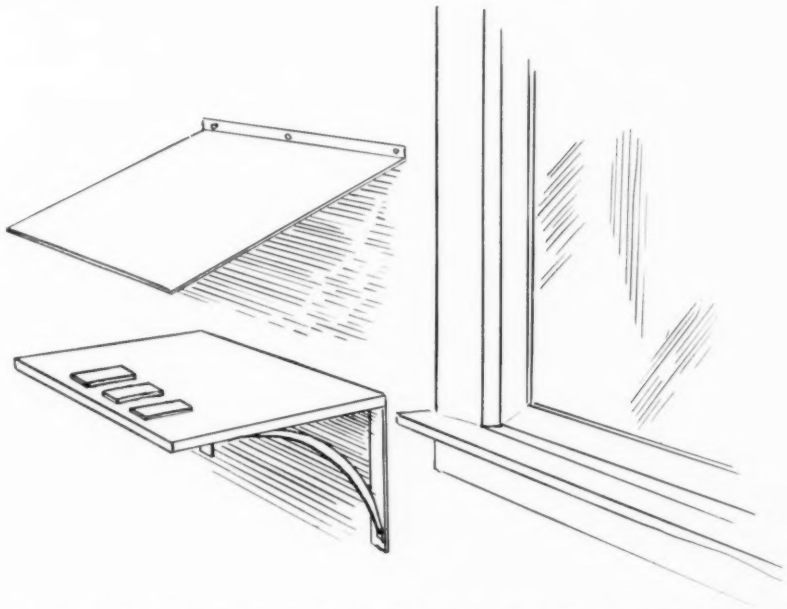

Fig. 1.—Shelf with cover, such as was used for exposure of slides to atmospheric pollen.

I encountered certain difficulties at the Vaughan-Graham Clinic, in Richmond, Va., in pollen counts made on a group of slides from the Binghamton, N. Y., area. A number of the slides had dried out, others had been exposed in the rain, and still others, due to the position in which they had been placed, released an excessive amount of mineral oil on one or the other side.

I then became interested in developing a method that would avoid these conditions and would, in addition, facilitate the identification of the pollen grains. Consequently, I tried a number of materials, including mineral oil, glycerin, white vaseline, corn oil, almond oil, glycerin jelly, and cedar oil.

When vaseline was employed, it was smeared over the middle third of the slides and melted slowly over a Bunsen flame in order that a smooth surface might be obtained. The oils were applied to the slides without the aid of heat.

The cedar oil was that used with the oil-immersion lens. Some of it was found to be rather thick at times, and the addition of several drops of xylene was necessary to thin it. The oil was applied to the slide (which had previously been cleaned with alcohol) with the end of a glass rod, using only a small drop for each preparation. The rod was then moved horizontally on the slide

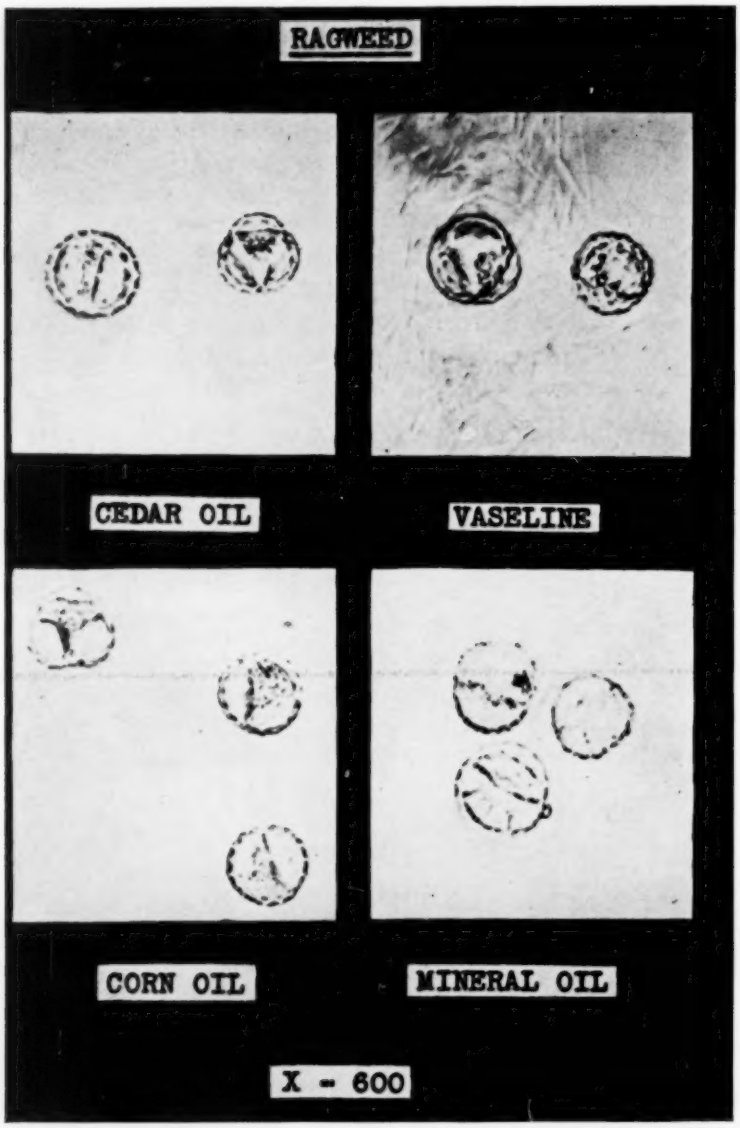

Fig. 2.—Photomicrographs of ragweed pollen grains showing comparison of cedar oil, corn oil, vaseline, and mineral oil.

to facilitate an even distribution over the middle third. Slides which we would probably want to file and store we protected with a cover slip. The slides were placed side by side on a shelf that extended about 18 inches beyond a window ledge. This shelf was protected from the rain by a metal cover suspended about 15 inches above, where it was attached to the side of the building (Fig. 1). The slides were observed before and after the mounting. Those for which cover slips had been used were found to be more satisfactory for study. All slides with the oil preparations presented a sharper distinction of the pollen morphology than did those with vaseline and plain glycerin jelly (Figs. 2 and 3).

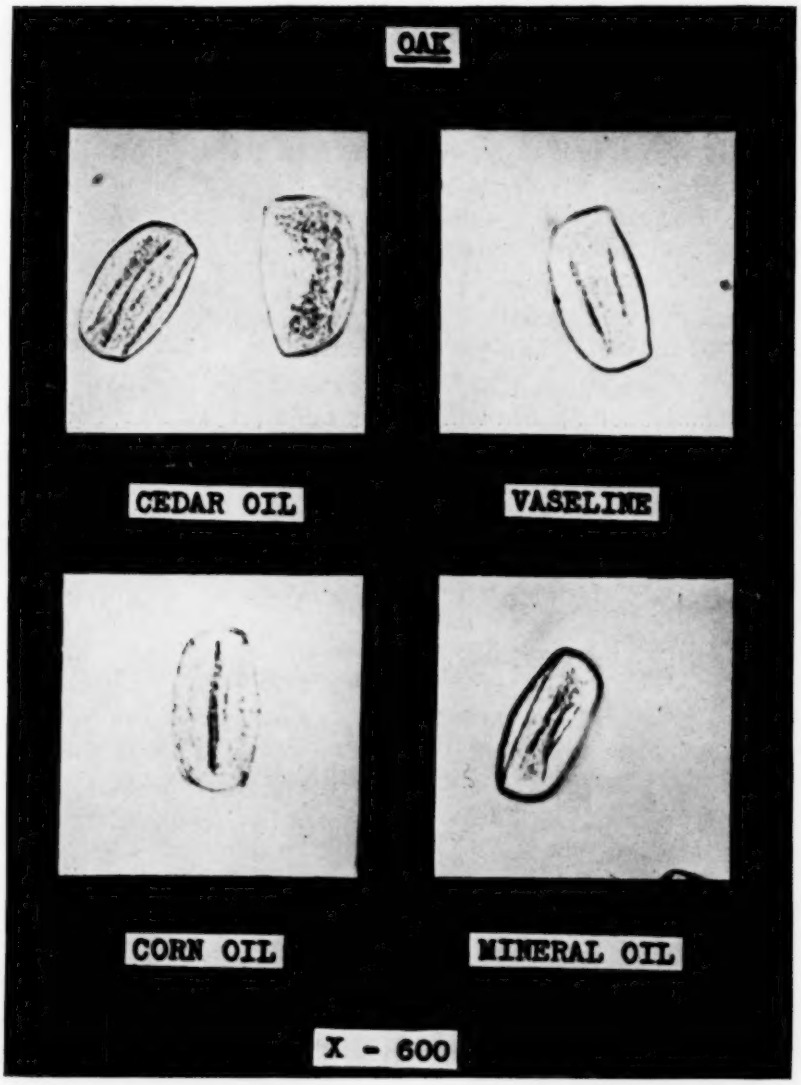

Fig. 3.—Photomicrographs of oak pollen grains showing comparison of cedar oil, corn oil, vaseline, and mineral oil.

The cedar oil preparations were found to be the most satisfactory from all points of view. As compared with vaseline, the cedar oil made the pollen grains more translucent on microscopic examination, while an amorphous or crystalline area was apparent in the slides prepared with vaseline, due to its cooling in preparation. After the cedar oil slides were exposed for a period of twenty-four hours, the oil would at times set (if the weather was very warm). From the beginning of the exposure it retained its adhesive qualities, which were more pronounced than the other oils.

All the other oils were found to be rather thin and tended to extend beyond the area over which they were applied. Later, when they were placed in a dust-proof slide box, they settled more readily to the side of the slide that

was down than did the cedar oil. The glycerin jelly preparations lacked the adhesive quality that was found in the slides with thin films of cedar oil and were not as translucent. The glycerin jelly was heated each time before the cover slip was mounted.

Plain glycerin jelly and glycerin jelly containing various stains in different concentrations were studied, but none of the stained preparations were found to be superior to the plain glycerin or as good as the oil preparations for routine work; however, some of the stained preparations showed more detailed structure.

Both sheltered and unsheltered slides were exposed at the same time, but there was little difference in the total pollen counts.

Cedar oil slides may be filed in dust-proof slide boxes following their exposure to atmospheric pollen without any other preparation; however, I did mount some of the cedar oil preparations with Canada balsam and cover slip. This did not interfere in any way with the distribution of the pollens on the slides. A further advantage is that they may be kept in this manner for future reference without being exposed to contamination with dust.

SUMMARY AND CONCLUSIONS

1. Comparative studies were made of atmospheric pollen slides prepared with glycerin jelly, glycerin, vaseline, corn oil, almond oil, mineral oil, and cedar oil.

2. Glycerin jelly containing stains presented an advantage over plain glycerin jelly where study of the structural detail was desired, but not for routine use.

3. Cedar oil was found to be preferable to the other oils in that it remained in the area over which it was applied and did not spread or run off the sides of the slide following exposure to atmospheric pollen. Cedar oil appeared to be more adhesive than some of the other preparations.

4. Cedar oil made the pollen grains more translucent to the microscope light than did either vaseline or glycerin jelly.

5. Cedar oil preparations may be filed, either mounted with Canada balsam and cover slip or unmounted, in dust-proof slide boxes.

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THE VENTILOGRAPH: AN IMPROVED RECORDING VENTILOMETER AND ITS APPLICATIONS

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TWO years ago one of us described a recording ventilometer[1] which, as an auxiliary unit attached to the familiar Benedict-Roth metabolism apparatus, enables recording on the kymograph chart, in addition to the customary spirogram, a second curve which by analogy is termed the *ventilogram*. The spirogram by its slope denotes the amount of oxygen absorbed by the patient per unit of time. The ventilogram serves as an indication and measure of the rate and amount of pulmonary ventilation during the corresponding period.

Since the publication of the first paper, the ventilograph, as this new unit has been termed, has been used experimentally in several research centers. Based on the experience thus accumulated, a new and improved model has been developed. It is the purpose of this paper to describe the improvements and to comment on the various uses to which this apparatus may be put.

The ventilograph consists of a mechanism that actuates a recording pen moving parallel to the pen which records the spirogram. The ventilograph pen is elevated one-twenty-fifth of the distance the spirographic pen moves during inspiration only. Since oxygen consumption is volumetrically about one-twenty-fifth of the pulmonary ventilation, the spirogram and ventilogram tend to follow substantially parallel courses, as shown in Fig. 2.

An assembled view of the mechanism of the ventilograph head is shown in Fig. 1. *f* is the pulley support arm of the standard Benedict-Roth metabolism apparatus. *A* is the original pulley wheel, and *a-a* is the original pulley chain, one end of which is attached to the oxygen bell and the other to the spirographic pen. This wheel *A* moves back and forth as the bell rises and falls. To the original fork *f* is added a support for the gear wheels, and an extended shaft for the second pulley *B*. Within the gear case *G* is a beaded hard fiber wheel which, through the action of two blade-type pawls, is permitted to move in one direction only. One pawl is attached to the pulley wheel *A* and advances the fiber wheel during the inspiratory excursion of the bell. A second pawl attached to the frame holds the fiber wheel stationary during the reverse excursion of *A*. The unidirectional movements of this fiber wheel are communicated through a train of reduction gears to the second pulley wheel *B*, which has the same diameter as *A*. Attached to the periphery of *B* by the set screw *s* is a chain *b*; the rotation of *B* in the direction of the arrow thus serves to elevate the pen of the ventilograph which is attached to the lower end of chain *b*. This pen records

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the ventilogram, a line which by its slope denotes the sum total of the inspiratory excursions of the bell on a 1/25 scale. A safety device is incorporated in the gear train to prevent overwinding. The ventilograph pen may be reset to any desired position by pulling sleeve *r* outward to disengage the gears.

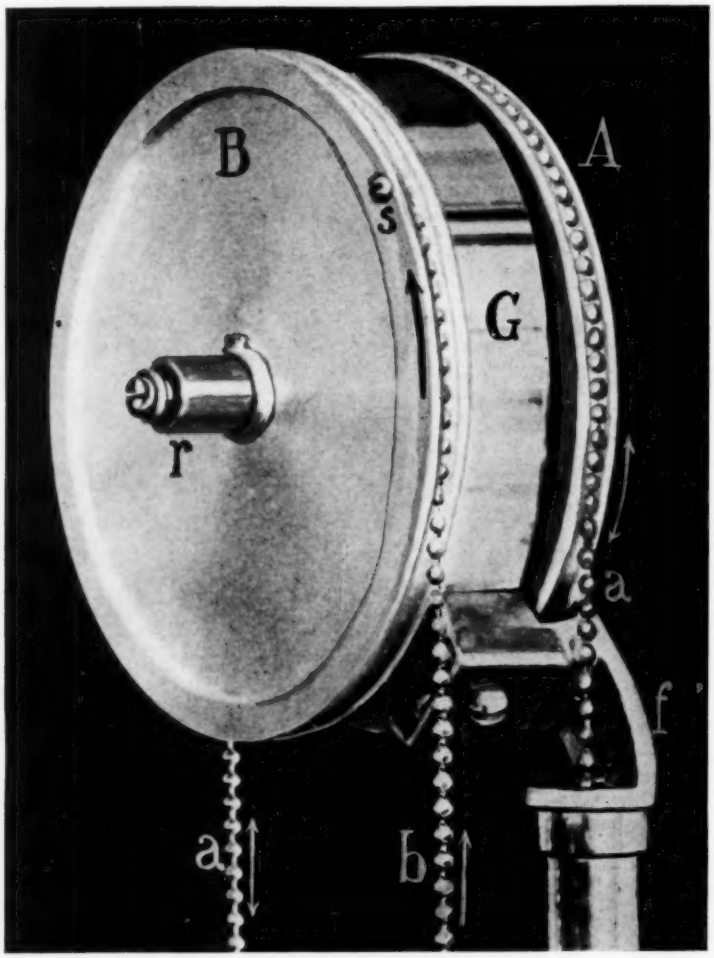

Fig. 1.—The pulley wheel assembly of the ventilogram. *f* is the fork of the stock model basal metabolimeter, supporting *A* the original pulley wheel of the machine. Chain *a* runs on the periphery of this wheel and makes reciprocal excursions with the oxygen bell, recording the familiar spirogram of oxygen consumption. A set of gears within the case *G* activates the added wheel *B* so that it moves in the inspiratory direction only, raising the chain *b* fixed at the screw *s* at one-twenty-fifth the amount of the inspiratory phases of *A*. *r* is a manual reset mechanism.

In the first model of the ventilograph, the unidirectional wheel was of bakelite and had a knurled periphery upon which two pawls bore at an angle, the combination acting as a ratchet in the ordinary sense. Although the wheel was given a very fine knurling (0.02 inch), it was discovered that when respiratory excursions were small, the error introduced by the failure of the pawls always to drop exactly into one of the spaces in the knurling might amount to as much as 5 per cent, though ordinarily it did not exceed 2 per cent with human beings.

In the present model the ratchet mechanism was modified by giving the wheel two peripheral beads, with a modified pawl resting on each. By proper choice of the wheel material, and by selecting a pawl blade of appropriate springiness, sharpened and mounted at a certain angle, we found that the device acted as a true ratchet, although there are no "teeth" on the periphery of the wheel. Provision has also been made for the adjustment of the tension of the pawl blades against the wheel by means of conveniently accessible screws.

By actual measurement the loss has been reduced to a point below the error of available checking means, substantially less than 1 per cent. In addition, two further advantages have been gained. Through the elimination of the ratchet teeth, the device has been made silent in operation, and since the pawl blades are more flexible, and do not have to lift over a series of teeth, the friction introduced, though scarcely appreciable before, has been reduced to one-third or one-fourth of its former value.

The ventilograph has proved a great timesaver wherever the determination of pulmonary ventilation has been desired in the course of spirometric experiments using closed circuit metabolism apparatus as such or in modified form. With such apparatus, the only previous methods of estimation were the measurement of the cumulative length of successive inspiration lines on the kymographic record, either by calipers or by a map tracer. Both methods are fatiguing, time-consuming, and subject to considerable possible error.

A further advantage of the ventilograph is that the slope of the ventilogram may be used, even for periods of less than a minute, as an index of the *rate* of ventilation, as distinguished from the *amount* of ventilation over a longer period. It is probably the only satisfactory means of estimating ventilation rate for short periods, for while the general appearance of the spirogram (i.e., the depth of respiration and the rate) furnishes a rough indication, the difficulty of mentally integrating both those factors is obvious, as is its measurement from the spirogram as previously described.

The use of the ventilograph to determine ventilation rates is illustrated in Fig. 2. The graph is recorded from right to left. Upstrokes of the spirogram indicate inhalation; downstrokes, exhalation. A given point on the ventilogram corresponds to a point on the spirogram one space to the left, since the pens record that distance apart. Vertical lines represent minutes.

To determine the ventilation rate for a given portion of the ventilogram, a line is drawn paralleling that portion in the same manner as in oxygen determination from the spirogram. This line may be as long as necessary to enable accurate measurements of its rise in a given number of minutes. From the figure thus obtained, the rise per minute is calculated and multiplied by 0.52, which factor yields the ventilation rate in terms of liters per minute.

For instance, line AA' represents the initial slope of the ventilogram, during the period of quiet breathing indicated on the spirogram. The line rises 50 mm. in four minutes, or 12.5 mm. per minute. Multiplying 12.5 by 0.52 gives the ventilation rate, 6.50 liters per minute. Similarly, line BB' represents an in-

creased ventilation rate produced by more rapid breathing during a one-half minute period. The line rises 56 mm. in two minutes, or 23 mm. per minute. Similarly, line *CC'* represents a still higher ventilation rate of 25.45 liters per minute.

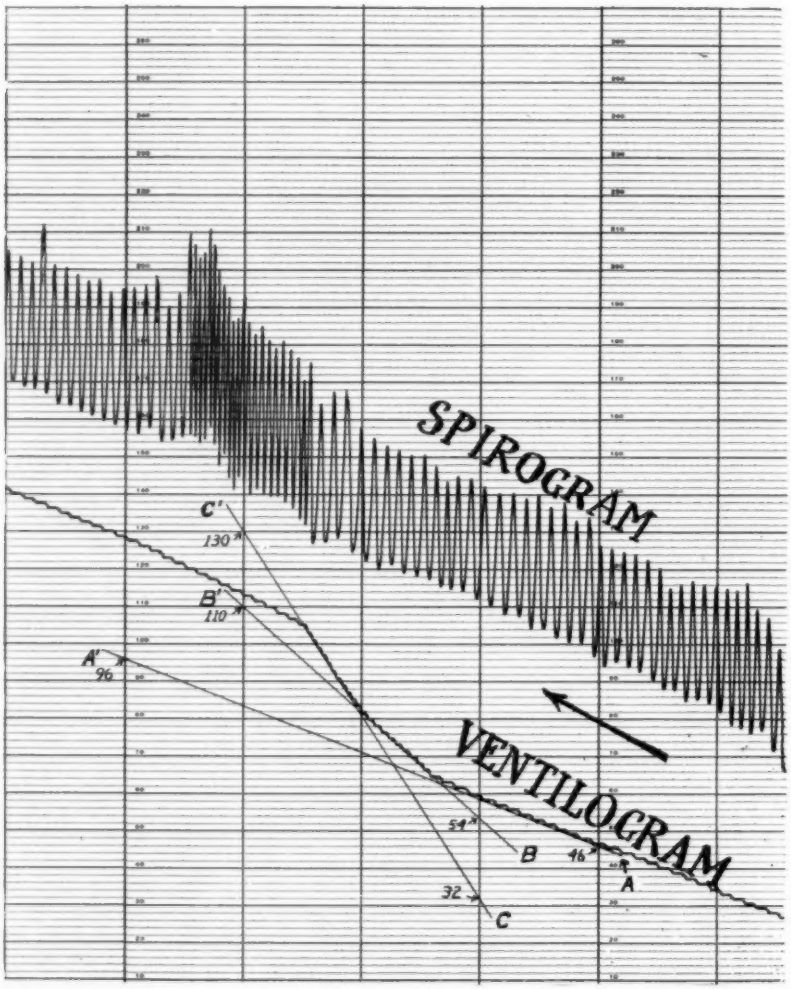

Fig. 2.—The spirogram is recorded by the pen attached to the spirometer bell by a chain passing over the original reciprocal pulley (Fig. 1*A*). The ventilogram is recorded by a pen attached to chain *b* (Fig 1). This is raised by pulley *B* through a distance equaling one-twenty-fifth the total inspiratory excursions of the spirogram. The spirogram gives both total ventilation and, by extension of sections of the curve, ventilation rates for selected periods. Since the pens cannot be superimposed, any point on the spirogram has a known constant time lag on the ventilogram curve—in this case exactly one minute.

The ventilograph was first designed to facilitate the investigation of the "oxygen-absorbing power" index proposed by Ziegler[2] and the similar "ventilation equivalent for oxygen" index proposed by Moncrieff.[3] Since then it has found other applications. Most of these are as yet in the research field, but experience makes it likely that clinical apparatus embodying the ventilograph will shortly be available.

Cournand and Richards[4] have developed an apparatus of a modified Benedict-Roth type, with ventilograph and other accessory equipment, for testing respiratory functional efficiency in chest diseases and other conditions.

Barach has used ventilograph-equipped spirometers in research on various phases of the diagnosis and treatment of asthma and emphysema,[5] in the study of the effect of oxygen and helium therapy on respiration[6] and in a field of research as yet relatively untouched in this country, bronchoscopic spirometry or "bronchospirometry," in which the air from the right and left lungs is separately conducted through a special bronchoscope to individual spirometers.[7] Pinner[7a] is also using similar equipment for bronchospirometry in clinical practice.

Recent work by Landt and Benjamin[8] indicates the possibility of studying cardiac functional capacity through the response of pulmonary ventilation to progressive lowering of the oxygen content of the inspired air. The ventilograph is being used in further research in this field. It is possible that similar application may be made in apparatus for the "metabolic exercise tolerance test" for patients with cardiac disease, proposed by Soskin and coworkers.[9]

Work is currently in progress at the Pierce Laboratory for Hygiene at Yale University, for the determination of the physiologic effects (including effects on metabolism and respiration) of various conditions of temperature, humidity, and radiant energy in the environment.[10] Here again a ventilograph-equipped metabolism apparatus is an essential part of the equipment. Similar apparatus is in use at the Aero-Medical Research Laboratory at Wright Field, where it is applied in some phases of aviation respiration research, not yet completed.[11]

Since the publication of the work of Nielsen and Roth[12] on the significance of various types of spirograms, little has been published on the subject of clinical spirography. A recent renewal of interest has occurred, particularly in the field of clinical psychiatry, and it appears likely that with the availability of a convenient means of studying the pulmonary ventilation rate, this factor will be considered as an adjunct to the appraisal of the spirogram proper. Investigation along this line is currently being initiated at the Massachusetts General Hospital in Boston[13] and the Presbyterian Hospital in New York.

SUMMARY

A new and improved model of the ventilograph, a means of graphically recording the pulmonary ventilation with closed-circuit recording spirometers of the Benedict-Roth type, is described, and its applications in the field of research and clinical medicine are discussed.

The ventilograph may be obtained on special order from the makers, Warren E. Collins, Inc., 555 Huntington Avenue, Boston, Mass.

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AN APPARATUS FOR THE MANUFACTURE OF WAX PARAFFIN AMPOULES FOR SILVER NITRATE SOLUTION USED IN THE PREVENTION OF OPHTHALMIA NEONATORUM*

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THE preparation and distribution of silver nitrate solution for the prevention of ophthalmia neonatorum has become a routine procedure of many public health laboratories. A method for the manufacture of ampoules composed of a mixture of beeswax, paraffin, and paraffin oil, for the distribution of this product, was originally described by Young.[1] Silver nitrate solution stored in these ampoules often deteriorated rather rapidly, resulting in the formation of a black precipitate and a strongly acid solution. Bunney[2] showed that the beeswax was responsible for this deterioration, and solved the problem by making a paraffin ampoule and coating it with beeswax. In such ampoules the silver nitrate solution never comes in contact with the beeswax—which is essential to prevent cracking or crumbling—and, therefore, is not subject to the deleterious effects of this material.

Bunney's method consists essentially of first dipping brass spindles twice in a melted mixture of 12 per cent paraffin oil and 88 per cent paraffin of 68° to 72° C. melting point. The spindles are then dipped several times in a melted mixture containing 71 per cent beeswax, 8 per cent paraffin oil, and 21 per cent paraffin of 56° C. melting point. The ampoules are filled with silver nitrate solution and sealed by running melted paraffin into them on top of the silver nitrate solution.

*From the Bureau of Laboratories, State Department of Public Health, Montgomery.
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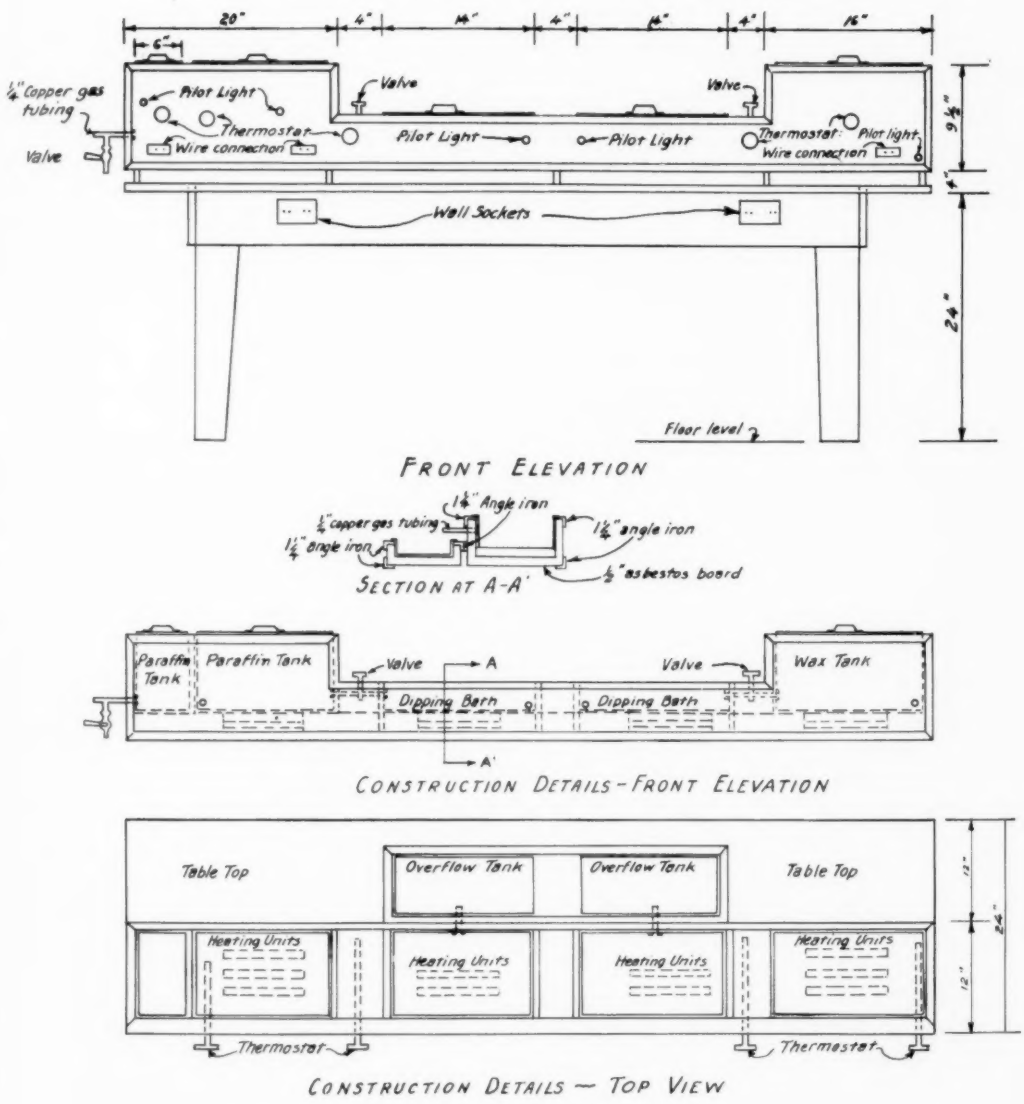

Fig. 1.—Paraffin wax bath.

An apparatus designed to facilitate manufacture of paraffin-lined beeswax ampoules of the type described by Bunney is outlined here. These ampoules have been made in this laboratory for two years, and the following problems were encountered in the use of the old type of apparatus:

1. It was necessary to have three melting baths; one for the 68° to 72° C. melting point paraffin; one for the beeswax-paraffin mixture; and one for the paraffin used for sealing.

2. Maintenance of the beeswax mixture and the high melting point paraffin at a constant depth in the dipping tank.

3. Proper control of the temperature of the two tanks of paraffin and the beeswax at their respective melting points so that ampoules of uniform thickness could be made.

4. Arrangement of the different units so that the whole process could be carried on with a minimum number of workers.

The apparatus shown in Fig. 1 was designed to overcome these difficulties in manufacture. The unit* consists of three melting tanks, two dipping vats, and two overflow pans, each of which is equipped with separate heating units. The three melting tanks and the two dipping vats are each equipped with separate thermostats, so that each can be kept at the exact temperature desired. Each vat has an overflow pipe located in the side, and at such distance from the bottom that the beeswax and paraffin can be maintained at a constant level, thus assuring ampoules of uniform length. Excess wax or paraffin flowing from the melting tank to the dipping vat runs through the overflow pipes into the pans, where it can be kept melted and returned at intervals to the melting tank.

On the end of the assembly is located the melting tank for paraffin used for sealing the filled ampoules. The melted paraffin is dispensed through the hand-operated metal valve at the end of the tank. The apparatus is assembled in a framework of angle iron and asbestos board, mounted on a table of suitable dimensions and height. The tanks, covers, and finishings are of stainless steel. Most of the electrical wiring is concealed underneath the table top with short wires equipped with detachable connections coming from the sides of the table for attaching to the separate heating units. Electric current is provided by a single long extension cord that may be attached to any light socket or wall plug. The legs of the table are equipped with casters so that the whole unit can be moved easily for storage when not in use.

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*This apparatus was built by the Apparatus and Specialty Co., Lansing, Mich., for the Bureau of Laboratories of the Alabama State Department of Health.

A SIMPLE BLOWFLY CAGE FOR THE CULTURE OF SURGICAL MAGGOTS*

MAURICE S. TARSHIS, B.A., M.S., CORVALLIS, ORE.

IN A PREVIOUS article in this JOURNAL[1] a simple blowfly cage for the culture of surgical maggots was described. Since then another type of cage has been devised. This cage, which has proved very satisfactory, is much simpler in design and costs less to construct. It is hoped that it will prove useful not only to those interested in the culture of surgical maggots, but also to those interested in the rearing of the blowfly or of other flies for experimental studies.

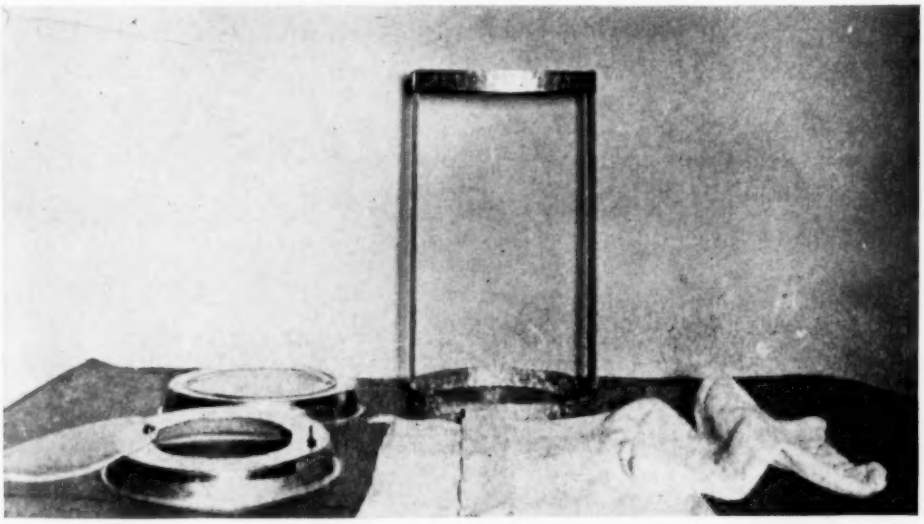

Fig. 1.—Framework and other parts of cage. Note construction of various parts.

The frame of the cage consists of two galvanized iron rims, 1 inch wide and 10 inches in diameter, connected by parallel wooden supports, 16 inches high and 1 inch wide (Fig. 1). These wooden supports are fastened to the inside of the iron rims by means of flat-headed bolts and nuts. If greater rigidity is desired, the number of wooden supports may be increased; however, two supports have been found to be sufficient. The edges of the iron rims are coated with liquid solder in order to facilitate placing the cloth cover on the cage as well as to obviate tearing of the cloth by jagged edges.

*From the Department of Zoology, Oregon State College, Corvallis.
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Fig. 2.—Showing arrangement of cloth cover. Note paper clips at top of cage and sleeve for entry into cage.

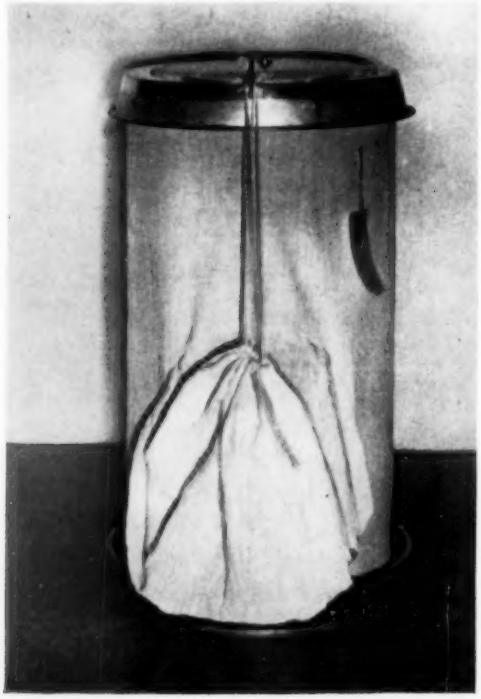

Fig. 3.—Complete arrangement of cage. Paper tag near top of cage is used for record. The tag is attached to the cloth cover by means of a small piece of flexible wire.

The cover of the cage consists of muslin sheeting. It is made to fit snugly around the frame. The cloth cover is allowed to extend for about an inch beyond the top and the bottom of the cage. To fasten the cover to the cage, the 1 inch border at each end of the cage is folded over each iron rim and held in place by means of paper clips (Fig. 2). A sleeve, 12 inches long and 6 inches in diameter, is sewed into this cover several inches from the bottom. A ¼ inch hem is made at the free end of the sleeve, with two small openings at opposite sides. Through this hem are drawn two strings, in the same manner as the draw strings of a purse (Fig. 2). The ends of each draw string are tied, and when the sleeve is not in use, the strings are attached to a small inverted bolt near the edge of the top of the cage. A nut holds this bolt in place (Fig. 3). The sleeve not only facilitates entry into the cage, but also prevents the flies from escaping during the placement and removal of food. A zipper opening makes it easy to arrange and remove the cloth cover. When the zipper is used, it is not necessary to make the sleeve, for by partly opening the zipper, entrance into the cage can be made satisfactorily. When a zipper is sewed into the cover, it should be placed so that it opens from the bottom to the top of the cage. In the placement and removal of food, however, the opposite side of the cage is illuminated near the top by means of a desk lamp. The light attracts the flies and obviates the danger of their escaping when the zipper is opened.

The cage is placed in a 10 inch metal pan, which serves as the bottom, and is covered by another 10 inch metal pan (Fig. 3). In each pan is cut a circular opening, about 8 inches in diameter, and each opening is covered with a 9 inch, transparent, celluloid disk. The celluloid disk for the pan to be used on the bottom of the cage is fastened to the pan with four small, flat-headed bolts and nuts. The disk for the pan to be used on the top of the cage is held in place with only one small, loosely screwed bolt and nut, so that it can be swung in and out of place. This arrangement permits the transfer of flies from soiled to clean cages.

To transfer the flies the clean cage is inverted over the soiled one, the loose celluloid disk on each cage is swung outward, and the bottom of the inverted cage is illuminated. The flies, being positively phototropic, are quickly attracted to the illuminated surface. When the flies have migrated to the clean cage, the celluloid disks are moved back in place, and the inverted cage is placed upright. The transparent disk also permits observation into the cage. This is especially convenient during the placement and removal of food dishes.

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A METHOD FOR THE DETERMINATION OF SUGAR IN SMALL AMOUNTS (0.02 C.C.) OF BLOOD*

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BUFFALO, N. Y.

DETERMINATIONS of the concentration of sugar in the blood are most conveniently made upon samples drawn from a vein. Such samples are usually so large that the analyst can make measurements with a fairly high degree of accuracy, i.e., 2 c.c. are measured with an accuracy of between 0.5 and 1 per cent. In a fair number of patients, particularly children, it is sometimes difficult, or practically impossible, to obtain this amount of material by a satisfactory venipuncture. To meet these difficulties a number of micromethods of analysis have been developed and adapted to the study of blood from the capillaries. In all methods of this kind there are inherent technical disadvantages. When a gravimetric method of measuring the sample[1] is employed, the cost of equipment and the special precautions necessary in the preliminary treatment make routine use quite unsatisfactory. In methods using an initial volumetric measurement of which several have been described,[2, 3] the decrease in accuracy of measuring the sample serves as one source of difficulty. This seems unavoidable, for the measurement of 0.1 c.c. with an accuracy of 1 per cent can be made only when great precautions in handling the material can be observed, and such precautions cannot be taken when working with blood oozing from the finger or ear. In our experience another difficulty was encountered when an attempt was made to apply these methods to the study of patients. Not infrequently it has been found almost impossible to obtain a suitable 0.1 c.c. sample from the usual puncture wound if the clinician or technician has not acquired considerable practice in the manipulations involved. It seemed to us that it might be worth while to investigate the effect of decreasing the size of the sample taken for analysis, and, even at the expense of an increased inaccuracy of the initial measurement, to decrease the amount of technical skill necessary for obtaining the material. Since analyses of 0.02 c.c. of blood obtained from a superficial puncture of the finger or ear are commonly made in the determination of hemoglobin, and since many, if not all medical students, interns, and technicians are thoroughly drilled in the technique of making such estimations, an attempt was made to see whether this amount of blood would serve for a fairly satisfactory determination of the blood sugar concentration.

*From the Laboratory of the Buffalo General Hospital and the Department of Pharmacology, University of Buffalo, School of Medicine, Buffalo.
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Since many clinical laboratories have found that colorimetric methods are satisfactory for diagnostic purposes, attention was centered upon developing a colorimetric procedure. Folin and Malmros[4] have described a modification of the method of Hagedorn and Jensen[2] in which 0.1 c.c. of blood from the finger is used, and the final estimation is based upon the determination of the amount of Prussian blue formed under standard conditions. The intensity of the final color obtained in this procedure, and the relative stability of the oxidation-reduction system employed in it, made it seem probable that relatively slight modifications in the technique would make it possible to analyze even smaller amounts of blood in a similar way. A study was, therefore, undertaken, and the method described below, which we believe is adapted to the study of finger blood for diagnostic purposes, was developed.

Apparatus.—(1) A pipette of fine capillary tubing, graduated at 0.02 c.c. The Sahli pipette for the estimation of hemoglobin was used for this purpose.

(2) A microcolorimeter, capable of measuring the amount of color in 1 or 2 c.c. of solution.

(3) Small tubes, approximately 3 inches in length and 0.5 inch in diameter, such as are used for serologic tests. A water bath, standard pipettes, and flasks, such as are used in routine work complete the necessary equipment.

Reagents.—(1) Dilute tungstic acid. Measure 5 c.c. of 10 per cent sodium tungstate into a 250 c.c. volumetric flask. Add water to give a volume of about 200 c.c. Add, while shaking, 5 c.c. of 2/3 N sulfuric acid, and dilute to 250 c.c. with distilled water. This reagent, which is the one recommended by Folin and Malmros for the precipitation of protein, must be prepared freshly every two weeks. It should contain no measurable amount of reducing material, i.e., it should give no color when a blank analysis is carried through the procedure.

(2) Potassium ferricyanide. Dissolve 0.05 Gm. of pure potassium ferricyanide in 100 c.c. of distilled water. This reagent should be discarded when a precipitate forms, or when a blue color develops upon the addition of the iron solution.

(3) Sodium carbonate. Dissolve 1.6 Gm. of anhydrous sodium carbonate in 100 c.c. of distilled water. The ferricyanide and carbonate solutions have been somewhat modified from those described by Folin and Malmros.

(4) Ferric iron solution. Dissolve 0.10 Gm. of ferric ammonium sulfate in 50 c.c. of water. Add 5 c.c. of syrupy (85 per cent) phosphoric acid and 0.30 Gm. of "duponol."* The solution is made to a volume of 100 c.c.

(5) Standard glucose solution. A stock solution containing 0.2 per cent of pure glucose in water is prepared. This is covered with a thin layer of toluol and kept in the refrigerator. The standard used in the determination is prepared from this stock by diluting 1 c.c. of it to 200 c.c. with distilled water. This dilute standard must be prepared fresh every day.

*"Duponol" is the trade name for an emulsifying agent prepared and sold by E. I. DuPont de Nemours & Co. It is added to make the suspension of Prussian blue more permanent. We encountered some difficulty in preparing the gum solution recommended by Folin and Malmros for this purpose, and have, therefore, substituted "duponol" in their procedure. This preparation, in the amount used, maintained the Prussian blue in clear solution for between twenty-four and forty-eight hours.

Method.—Measure 2 c.c. of the tungstic acid solution into a small test tube. The blood, either capillary blood from a puncture wound or oxalated blood, is drawn exactly to the mark in a Sahli pipette, and any excess is wiped off the stem. The blood is then quickly expelled into the tungstic acid solution. It usually tends to settle to the bottom, and the clear supernatant fluid is drawn two or three times into the pipette and returned to the test tube to insure thorough rinsing. The blood and the tungstic acid are then mixed well, and the tube is allowed to stand two or three minutes or more. It is then centrifuged. One cubic centimeter of the supernatant fluid is measured into the tube described above, and 1 c.c. of the diluted standard glucose is put into a similar tube. To each is added 0.5 c.c. of potassium ferricyanide, followed by 0.2 c.c. of carbonate. Both tubes are placed in a boiling water bath for eight minutes, and then cooled in running water. One cubic centimeter of the ferric iron solution is then added to each, and the colors compared after fifteen minutes or more in a microcolorimeter.

TABLE I

COMPARISON OF RESULTS OBTAINED BY THE FOLIN-WU METHOD AND THE MICROMETHOD

	FOLIN-WU METHOD	PROPOSED METHOD	DILUTION USED	DIFFERENCE PER CENT	DIFFERENCE
	mg./100 c.c.	mg./100 c.c.			mg./100 c.c.
	99	102	1:1	3.0	3
	96	103	1:1	7.3	7
	95	87	1:1	8.4	8
	80	91	1:1	13.7	11
	133	118	1:1	11.3	15
	165	145	1:1	12.1	20
	392	380	1:4	3.1	12
	325	325	1:4	0.0	0
	103	105	1:1	1.9	2
	143	133	1:1	7.0	10
	84	85	1:1	1.2	1
	580	616	1:4	6.2	36
	58	61	1:1	5.2	3
	75	78	1:1	4.0	3
	222	214	1:1	3.6	8
	200	210	1:1	5.0	10
	196	184	1:1	6.1	12
	215	228	1:1	6.0	13
	300	324	1:2	8.0	24
	304	291	1:2	4.3	13
	360	341	1:2	5.3	19
	292	315	1:2	7.9	23
	186	169	1:1	9.1	17
	460	489	1:4	6.3	29
	32	42	1:1	31.0	10
Mean	207.4	209.4			

When the glucose concentration is 50 to 200 mg. per 100 c.c. of blood, the method can be followed as described. For concentrations less than 50 mg. the interference of the yellow color makes the reading inaccurate. When the blood sugar concentration is higher than 200 mg. per 100 c.c., the supernatant liquid must be diluted before analysis. When the concentration is between 200 and 400 mg., satisfactory results are obtained if the filtrate is diluted 1:2 or 1:4 with distilled water; blood sugar concentrations between 400 and 800 mg. require dilution of 1:4 or 1:8. If the readings are kept within this range, it is not

necessary to use a light filter to prevent significant interference by the yellow color of the unreduced potassium ferricyanide.

Calculations.—$\frac{S}{U} \times$ Standard $\times$ 1.01 = Mg. glucose per 100 c.c. of blood.

Where S = reading of standard, U = reading of unknown, and standard = 100 mg./100 c.c. If the filtrate was diluted 1:2 or 1:4 or 1:8, the results must be multiplied by the appropriate factor—2, 4, or 8 as the case may be. The standard is so diluted that the value is 100 when the colors of the standard and the unknown are alike, and the procedure is carried through upon the undiluted supernatant fluid as previously described. The factor 1.01 is used because the total volume of blood plus tungstic acid solution is 2.02 c.c., and the amount of blood contained in it is 0.02 c.c. A simplified expression of the formula is $\frac{101S}{U}$. Dilution of supernatant fluid = Mg. glucose per 100 c.c. of blood.

The method was standardized by making simultaneous determinations by the method of Folin and Wu[5] and by the technique described upon samples of oxalated venous blood. The results are presented in Table I, in which both the actual and the percentage differences between the two values are given. It is evident that in spite of the errors inherent in measuring such small quantities of blood, the agreement is close enough to suggest that the method should be satisfactory for diagnostic purposes. The percentage difference between the two methods has been summarized in Table II.

TABLE II

PER CENT DIFFERENCES BETWEEN THE RESULTS BY THE FOLIN-WU METHOD AND THE MICRO-METHOD

PER CENT RANGE	NUMBER
0 to 5	9 pairs of values
5.1 to 10	12 pairs of values
10.1 to 15	3 pairs of values
Over 15	1 pair of values

If the method is applied to diagnosis, however, there are certain facts which should be kept carefully in mind. Such a method cannot be considered as a satisfactory substitute for the standard analytical procedures upon venous blood, for it is not possible to obtain the same degree of accuracy in measuring very small amounts as in measuring larger ones. The nature of the difference between capillary and venous blood must also be kept in mind when the results are interpreted. In the fasting condition it appears that there is no significant difference between the blood sugar concentrations in the two parts of the circulatory system,[6] but after the ingestion of carbohydrate this difference is often large[6-9] and an interpretation of results obtained upon such material in terms of normal values based upon analyses of fasting venous blood might easily lead to mistakes in diagnosis. Conservative interpretations must certainly be made after food has been ingested.

A method for the determination of sugar in very small samples of blood (0.02 c.c.) is described. The method is based upon a modification of the technique of Folin and Malmros. The results obtained agree closely with those of duplicate analyses by the method of Folin and Wu.

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A COMPARISON OF METHODS USED IN THE DETECTION OF THE SICKLE-CELL TRAIT*

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METHODS have been advocated for the detection of the sickle-cell trait. These include the following:

1. *Moist Preparation* (Emmel, Quoted by Cook and Meyer,[1] and Emmel[2]). A drop of capillary blood is sealed under a cover slip and examined microscopically at intervals thereafter. A change of shape of the erythrocytes from discoid to bizarre "fish-fin" and multipointed forms is diagnostic of sicklemia or the sickle-cell trait. The moist preparation has been widely adopted and is the method most widely used in diagnosis and in surveys of the sickle-cell trait.

2. *Gas Chamber Method* (Hahn and Gillespie[3]). A suspension of blood in citrated saline is placed on a cover slip and inverted over a small gas chamber. The edges are sealed. Carbon dioxide is passed through the chamber, and the shape of the cell is observed microscopically.

3. *Moist Stasis Preparation* (Scriver and Waugh[4]). A rubber band is placed around the proximal portion of the finger and allowed to remain for five minutes. The distal end of the finger is then punctured, and a drop of the dark blood is sealed under a cover slip (Fig. 1). Scriver and Waugh noted that the red blood cells sickled more rapidly when collected under such anoxemic conditions than when the blood was collected without stasis.

4. *Test Tube Method* (Beck and Hertz[5]). A drop of finger blood is allowed to fall in a saline citrate solution in a test tube. Paraffin oil is added to prevent exposure to air. The preparation is allowed to stand for twenty-four hours at

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room temperature. Formaldehyde, 10 per cent in normal saline, is then added to fix the cells and to prevent their reversion to the round form on exposure to air. Moist preparations of the fixed cell suspension are examined microscopically.

5. *Moist Preparations Plus Dyes and Other Chemicals* (Hansen-Pruss[6]). Clean slides are covered with a thin film of chemical in solution, and the film is allowed to dry. A drop of blood from the finger is taken on a clean cover slip and placed on the prepared slide. The preparations are sealed with vaseline and examined microscopically.

Hansen-Pruss noted that the erythrocytes sickled faster and that the number of positive cases detected in an unselected group of negro patients was greater when brilliant cresyl blue, Janus green, methylene blue, and sodium cyanide were used than when the blood was untreated or when neutral red was used.

Because of the various methods advocated and the lack of evidence supporting the superiority of one method over another, a comparative study of the various methods used for the detection of the sickle-cell trait was made.

GAS CHAMBER METHOD

The gas chamber method is an ingenious method for the study of the factors involved in the sickling of erythrocytes, but it is impractical as a routine procedure. Special equipment and skill in manipulation are required. In our hands, the gas chamber method was less reliable than the ordinary moist, stasis moist, and test tube methods. Graham and McCarty,[7] and Scriver and Cooley,[8] and others have likewise found the gas chamber method to be unsatisfactory.

MOIST NONSTASIS AND MOIST STASIS WITHIN ONE HOUR

Two moist stasis and two moist nonstasis preparations were made on 406 unselected colored patients at the John Gaston Hospital. The preparations were examined within one hour after being made. One hundred erythrocytes were counted in each preparation, and the percentage of sickled cells was noted. The moist stasis preparations revealed sickling within the first hour in 26 instances, whereas the moist nonstasis preparations revealed sickling in 10 instances. The percentage of sickled cells in the two preparations of blood taken from the same patients and made by the same technique varied. Great variations were noted in the number of sickled cells in preparations from different patients. The number of sickled erythrocytes was significantly greater in the moist stasis preparations than in the moist nonstasis preparations. The average percentage in the 26 positive stasis preparations was 35 per cent, whereas the average percentage of sickled cells in the 10 positive nonstasis preparations was less than 1. In no instance did the nonstasis preparation show sickling in excess of the stasis. From these observations it is concluded that the stasis moist preparation is superior to the moist nonstasis preparation when the readings are made within one hour.

MOIST NONSTASIS, MOIST STASIS, AND TEST TUBE METHODS IN TWENTY-FOUR HOURS

On each of the 406 unselected colored patients noted, a test tube preparation was made along with the two moist nonstasis and two moist stasis preparations. The technique as advocated by Beck and Hertz,[5] was followed as carefully as possible.

Of the 406 unselected cases, 37 were found to have erythrocytes possessing the ability to sickle in at least one of the preparations, an instance of 9.1 per cent. Of the 37 positive cases, the moist stasis was positive in 36 instances. With the test tube method the readings were positive in 35 instances. With the moist nonstasis preparations there were 31 instances of sickling (Table I).

The percentage of cells sickled in twenty-four hours in the stasis preparations was greater than in the test tube in 23 instances, less than in the test tube in 7 instances, and essentially the same in 6 instances. The average percentage of cells sickled with each method at the end of twenty-four hours is given in Table I.

TABLE I

METHODS	ONE HOUR		24 HOURS	
	INCIDENCE	AVERAGE % SICKLING	INCIDENCE	AVERAGE % SICKLING
Stasis	26	35	36	71
Nonstasis	10	1	31	45
Test tube			35	46

In two instances in which the stasis moist preparation was positive and the test tube was negative, the percentage of sickled cells in the stasis moist preparations was 94 and 20. In one instance in which the stasis preparation was negative and the test tube was positive, the percentage of cells sickled in the test tube was less than 1 per cent.

From these observations it is concluded that the stasis moist preparation and the test tube methods are more reliable than the moist nonstasis method for the detection of the sickle-cell trait, and that the stasis moist preparation and the test tube methods are of about comparable reliability. On account of the simplicity of the stasis moist preparation in comparison to the test tube method and the fact that the reading in the stasis moist preparation can often be made immediately, the stasis moist preparation is considered to be superior to the test tube method as a routine procedure.

VITAL DYE, MOIST STASIS, MOIST NONSTASIS, AND TEST TUBE METHODS

An attempt was made to repeat the work of Hansen-Pruss[6] and to compare the stasis, nonstasis, and test tube methods with the vital dye moist preparation method. A comparison was also made between the stasis vital dye moist preparation and the nonstasis vital dye moist preparation (Table II). The vital dyes were prepared and placed on the slides as outlined by Hansen-Pruss, except that 95 per cent alcohol instead of absolute alcohol was used. The moist preparations were examined microscopically immediately after returning from the ward to the laboratory, and a second reading made the next day. The test tube preparation was made with blood from the nonstasis finger, and the reading was made at the end of twenty-four hours.

In this series of experiments the blood of 51 unselected colored patients was examined; sickling was demonstrated in 8. The percentage of sickled cells in each preparation made from the 8 patients are given in Table II.

TABLE II

CASES	MOIST STASIS					MOIST NONSTASIS					TEST TUBE
	NO DYE	METH. BLUE	NEUT. RED	B. C. BLUE	JANUS GREEN	NO DYE	METH. BLUE	NEUT. RED	B. C. BLUE	JANUS GREEN	
Percentage of Erythrocytes Sickled Within 1 Hour											
1	90	20	25	0	20	0	0	0	0	0	
2	30	90	40	0	90	4	85	0	0	60	
3	91	90	91	0	75	4	30	3	0	6	
4	100	100	93	0	100	93	100	91	0	95	
5	10	1	1	0	0	0	1	0	0	0	
6	10	1	1	0	0	0	1	0	0	0	
7	70	65	60	0	60	10	6	3	0	6	
8	80	85	75	0	80	20	25	10	0	10	
Average	69	69	58	0	53	22	41	14	0	22	
Percentage of Erythrocytes Sickled Within 24 Hours											
1	92	96	94	94	90	54	96	0	0	35	85
2	93	98	50	15	97	30	93	H	1	93	94
3	92	H	93	0	H	74	85	H	0	0	0
4	100	100	95	0	100	95	100	94	0	100	79
5	85	81	82	0	H	84	89	56	0	H	80
6	80	88	93	0	70	75	80	70	0	70	75
7	80	80	80	0	70	75	80	70	0	70	75
8	100	100	95	0	90	100	100	93	0	90	70
Average	90	91	85	14	89	72	91	67	0.12	78	68

H = Hemolyzed.

A study of the findings in this table reveals that brilliant cresyl blue inhibits the sickling of erythrocytes. This is in agreement with our past experience with brilliant cresyl blue, for we have noted in hundreds of brilliant cresyl blue moist preparations of blood from patients with sickle-cell anemia in which the reticulocytes were being studied that the sickling was not as marked as it was in the moist preparations in which no dye was used.

The sickling was more marked in methylene blue moist preparations than in Janus green or neutral red moist preparations.

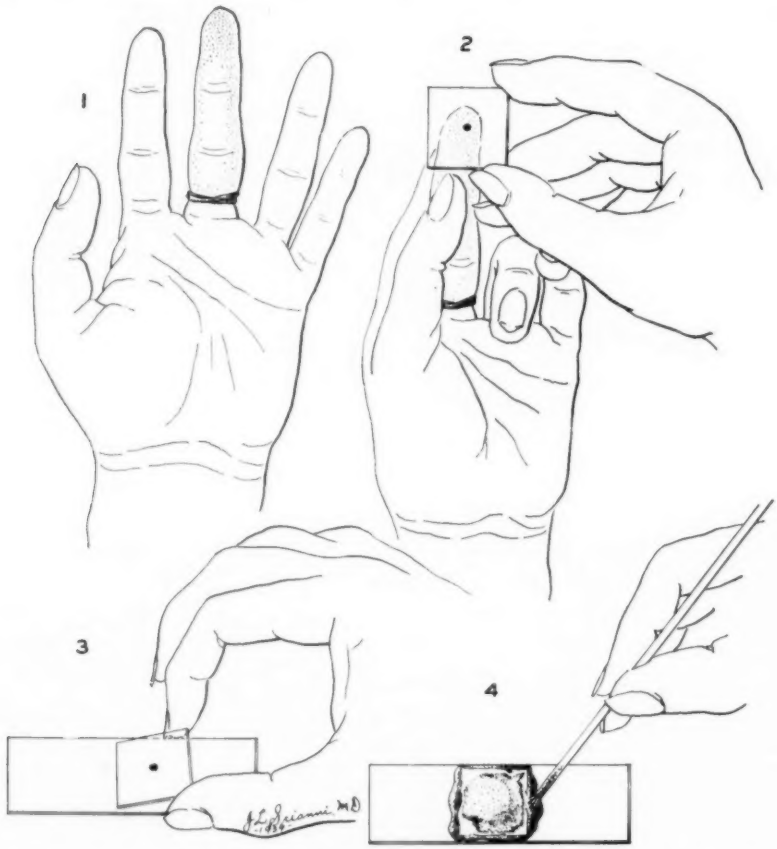
The stasis in the absence of dyes was more effective in producing sickling than dyes without stasis. The percentage of cells sickled in preparations with both dyes and stasis was no greater than in preparations with stasis in which no dyes were used.

The stasis method in this series yielded a greater percentage of sickled cells than did the moist nonstasis or the test tube methods.

COMMENT

The test tube method of Beck and Hertz yields sickled erythrocytes which are preserved in formalin and which are clearly defined and readily diagnosed. For the preservation of cells for teaching purposes, and for experimental studies of the effect of various substances on the sickle-cell phenomenon, the test tube method is recommended. As a routine method of diagnosis of sicklemia, however, the test tube method is inferior to the stasis moist preparation, for it is a little less reliable, and it is much more complicated, expensive, and time-consuming.

The use of dried films of dyes and other chemicals on slides as a simple means of studying the sickle-cell phenomenon and the factors that influence the sickling of erythrocytes is an excellent one. The use of methylene blue, Janus green, or neutral red as a routine procedure, however, has no apparent advantage over the stasis moist preparation; it has the disadvantage of being a more expensive and less practical method.



Figs. 1-4.—Stasis moist preparation for the detection of the sickle-cell trait.

The ordinary moist preparation is the simplest of all, but it is less reliable and requires a longer period of waiting before the reading can be made than does the moist stasis.

It is recommended that the moist stasis preparation be read whenever convenient after the preparation is made, but that the preparations in which the reading is negative within the first few hours be left for twenty-four hours at room temperature before the final reading is made. It is also advisable to make more than one moist stasis preparation, for sometimes the blood does not spread well, the seal is not complete, or the cells become hemolyzed. One should also transfer the blood from the puncture wound to the slide rapidly and prevent in so far as possible the exposure of the drop of blood to the air.

CONCLUSION

The most reliable and the most practical method for the detection of the sickle-cell trait which has yet been devised is the moist stasis method of Scriver and Waugh.

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THE USE OF A SINGLE ANIMAL FOR TESTING THE VIRULENCE OF C. DIPHTHERIAE*

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IN 1924 Fraser and Wigham[1] published a preliminary note on the use of intracutaneous tests on rabbits for determining the potency of diphtheria toxin and the virulence of diphtheria cultures. They reported that the variation between the reactions on individual rabbits was slight, and advocated the use of a dose of antitoxin five hours after the tests were placed, in order to preserve the animal at least until the completed reaction could be observed. A control test, using a known virulent strain, was included. They further recommended the use of the rabbit as assuring economy in time and animals, facility in reading reactions, and uniform basis for comparing several reactions on the same rabbit.

In 1926 Fraser and Weld[2] described a technique for intracutaneous testing on rabbits whereby a single animal could be used both as a test and as a control animal. This was accomplished by the intravenous administration of a large protective dose of antitoxin five hours after the test injections were placed, after which the test culture suspensions were injected into parallel skin areas on the other side of the rabbit. The rabbit was converted into a control by this dose of antitoxin, and the test was read by comparing the reactions on the two sides.

The use of the technique described by Fraser and Weld (1926) for determining the relationship between toxin production and intracutaneous virulence of recently isolated diphtheria cultures was reported in 1931 by

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Mader and Halpern.[3] These workers reported a variation of from 10 to 40 mm. in the diameter of the lesion in the cultures tested. They also used a known virulent strain as a control. This control culture, according to Mader and Halpern, yielded a "fairly constant reading between twenty-five and thirty millimeters in redness, hemorrhage and necrosis."

Fraser, Halpern, and Roy[4] in 1939 introduced another modification of the technique for the testing of virulence of diphtheria cultures by the intracutaneous inoculation of a rabbit. A mixture of culture suspension and antitoxin was inoculated into another skin area on the rabbit at the same time as the test culture suspension, an obvious saving in time and animals. A survey of diphtheria strains isolated from cases in California was in progress at the time this new modification of technique was described. Because many strains were to be tested for virulence and because white rabbits were available, the 1939 modification of the technique was adopted in place of the intracutaneous test on guinea pigs used in the department of hygiene since 1922[5, 6] for tests on both pure and field (mixed) cultures of diphtheria.

Pure cultures, isolated as single colonies from potassium tellurite-blood agar plates, were cultivated on Loeffler's medium. The growth, after forty-eight hours' incubation, was suspended in Stone's solution.[7] The use of this solution insures the retention of the toxin in the skin as it is formed. The culture suspension was divided and placed in two test tubes; to one portion diphtheria antitoxin was added (0.2 c.c. containing approximately 400 units for each 2.5 c.c. of test suspension), so that the mixture served as a control suspension; the unneutralized portion was the test suspension. One-tenth cubic centimeter of each portion was injected into rabbits by the intracutaneous route. The control was placed approximately 2 cm. below the test suspension. Female white rabbits were used so that the hair could be plucked from the sides, thereby insuring a relatively unirritated skin area for inoculation. This method for preparing the skin is superior to clipping, shaving, or the use of a depilatory for producing an area adequate in size and condition with the least effort on the part of the worker or discomfort to the animal. The tests were observed at twenty-four-hour intervals for four days. The occurrence of a red, indurated, and necrotic area at the site of injection of the test suspension, and of redness and induration only at the site of the injection of the test suspension, was interpreted as a positive reaction caused by the toxin produced by virulent diphtheria bacilli. The reaction was considered inconclusive when more induration, without necrosis, was observed in the test than in the control injection. A negative reaction was recorded when areas of redness and induration, which decreased in intensity after twenty-four hours, were noted at both injection sites.*

Since the morphologic characteristics and cultural reactions of all the cultures tested had been typical of true *C. diphtheriae*, the occurrence of negative and inconclusive virulence tests by this new technique indicated the need for retesting with the technique familiar to the workers in this laboratory. All cultures which had yielded inconclusive reactions produced positive re-

*The intracutaneous injection of Stone's solution alone will induce a red and indurated area which fades rapidly.

actions both on guinea pigs and on other rabbits when retested. Cultures that had yielded unquestionably negative reactions on rabbits were then included in the group, subject to retesting on other rabbits and guinea pigs. Upon retesting, some of these were positive on both test animals. Subsequently, several sets of tests, with freshly isolated strains, were done in duplicate on rabbits and singly on guinea pigs. It was observed that certain rabbits exhibited negative and inconclusive reactions to cultures which induced positive reactions on other rabbits and on guinea pigs. In order to discover what proportion of variation occurred in tests on rabbits a series of experiments were undertaken.

Test Cultures.—A set of 16 pure cultures of virulent diphtheria bacilli was selected; thirteen of these cultures had varied in reaction on two or more rabbits but had produced positive virulence tests on guinea pigs. Three cultures of the gravis type, which had always reacted positively on all rabbits and guinea pigs, were included as controls. Four gravis, five intermedius, and seven mitis strains were used.

Preparation of Culture Suspension.—In order to obtain a sufficient amount of suspension, several slopes of Loeffler's medium were inoculated with each test culture. This was accomplished by delivering, with a pipette, portions of a saline suspension of the growth on a Loeffler's slope onto the surface of each of several slopes. Complete seeding of the surface was accomplished by rotating the inoculum carefully before placing the tubes in the incubator. After forty-eight hours' incubation the growth on each slope was suspended in 2 c.c. of Stone's solution. A pool of the suspensions of each test culture was divided into two parts, to one of which diphtheria antitoxin was added as in the preliminary tests. A stained smear from each test culture was examined to confirm the presence of diphtheria bacilli in pure culture. Subcultures were made from one tube of each test culture in order to verify the viability of the inoculum. Because of the color and consistency of the suspending solution, only a rough estimation of the relative opacity of the suspensions could be made.

Test Animals.—Female New Zealand white, New Zealand red, and Himalaya rabbits from several sources were used. All were mature animals, weighing 2,500 Gm. or more. Considerable difference in age was evident; variation in skin texture was noted. White guinea pigs, weighing 300 Gm. or more, were used. Most of these were females. The skin areas on the rabbits were prepared by plucking as previously described. The guinea pigs were plucked and shaved.

EXPERIMENTS

Four groups of animals were tested at ten-day intervals. Each set consisted of 16 to 18 rabbits and 8 to 16 guinea pigs. In each experiment some animals received test suspension only, so that the reaction to cultures could be observed in the absence of antitoxin in the skin. Those injected with both test and control suspensions were designated as test animals. Some of these test animals were given the control suspensions on the same side and below the test suspension, while others were given the test inoculations on the right side and the control inoculations on the left side. Sixteen tests were placed on the rabbits, and 8 on the guinea pigs. All injections were

made by one person who had had sufficient previous experience to assure the uniformity of the inoculations. The injection site was changed in each set of tests to eliminate the chance of one culture being repeatedly placed in an unfavorable skin area. The reactions were observed daily for four days.

In the first series 3 of 5 control rabbits died before twenty hours had elapsed. Thereafter all control rabbits received a subcutaneous following dose of 1,000 units of antitoxin five hours after the test injections were placed. Despite the use of a following dose of antitoxin, all control guinea pigs of the first series died in less than twenty hours. A new lot of antitoxin was used in the next two groups, but in twenty-four hours the control guinea pigs were either dead or so ill that the tests could not be accurately read. In the last experimental group the control guinea pigs were given 1,000 units of antitoxin as a following dose. All these guinea pigs lived at least forty-eight hours.

Reactions were classified as positive when the central area of necrosis, which finally became scabbed, was 3 mm. or more in diameter. Reactions were classified as inconclusive when the necrotic area was less than 3 mm. in diameter, but the development of the lesion was at the same rate as positive tests on the same animal. Reactions were classified as negative when no necrosis was observed, or when no reaction could be detected after three days.

TABLE I

REACTION OF ANIMALS TO THE INTRACUTANEOUS INOCULATION OF A TEST SERIES OF CULTURES OF C. DIPHTHERIAE

	CONTROL ANIMALS		TEST ANIMALS		
	ALL REACTIONS POSITIVE	VARIABLE REACTIONS	ALL REACTIONS POSITIVE	VABIABLE REACTIONS	TOTAL
Rabbits	13	3	7	29	52
Guinea pigs	16	1	14	9	40

RESULTS

Fifty-two rabbits received intracutaneous injections of a set of pure cultures of diphtheria bacilli (Table I). Sixteen of these, serving as controls, received injections of test suspensions alone. All tests were positive on 13 of these rabbits, while 3 exhibited inconclusive or negative tests with culture suspensions that yielded positive reactions on other rabbits and on guinea pigs. Thirty-six rabbits were used as test animals, receiving both test and control suspension. Twenty-nine of the 36 rabbits showed inconclusive or negative reactions to the injection of test suspensions which induced positive reactions on control rabbits and guinea pigs, whereas only 7 reacted positively to all injections.

Tests on guinea pigs yielded more uniform reactions (Table I). Seventeen control guinea pigs were inoculated with test suspensions. On 16 all reactions were positive. Only one guinea pig showed variation in reaction to the inoculation of virulent diphtheria cultures. Twenty-three guinea pigs received injections of both test and control suspensions. Nine of these animals showed one

or more negative or inconclusive reactions to test suspensions which induced positive reactions in control rabbits and guinea pigs, while 14 reacted positively to all tests.

Two test strains (both intermediate type) frequently gave inconclusive or very small reactions on control animals. When test animals were injected with these strains, the reactions were usually inconclusive or negative, although large typical reactions were observed on a few test and some control animals. Two of the 3 gravis control strains were uniformly positive reactors on all animals tested. Each of the other 12 test strains reacted positively on some animals in an experimental group, but gave negative or inconclusive reactions on other animals in the same or another group.

Differences in size, age, breed of rabbit, skin area injected, or texture of the skin could not be correlated with the variation in reactivity of the individual rabbit to virulent diphtheria bacilli. Of the 29 test rabbits (injected with both test and control suspensions) from 2 to 14 tests were inconclusive or negative on a single rabbit. Marked differences in the rate of development of lesions on individual animals were noted. The differences in size and character of the lesions in their early stages were especially noticeable. The reactions on the control rabbits were uniformly and obviously larger than those on the test rabbits, though the same variation in rate of development and character of lesion was evident in individual rabbits in both test and control groups.

The reactions on control guinea pigs were larger than those on test guinea pigs. Variation in the rate of development in individual guinea pigs was noted, but there was less difference in the character of the lesion in guinea pigs than in rabbits. No difference in reaction was observed in rabbits or guinea pigs when the control suspensions were inoculated on the same side as the test suspensions or when all control suspensions were inoculated on one side.

DISCUSSION

Previously intracutaneous tests for potency of vaccinia virus (unpublished data) had shown such variation in rabbit reactions that these results with *C. diphtheriae* were not unexpected. It is evident that individual rabbits may fail to react to the injection of virulent diphtheria bacilli so that virulence tests on such animals might be falsely reported as negative. The use of a control test culture will not solve the problem since, in these experiments, only 2 of 16 virulent cultures reacted positively on all rabbits.

The use of one rabbit for a set of tests, which is made possible by the injection of a control suspension, evidently increases the hazard inherent in the use of rabbits as test animals. Only 7 of 36 rabbits yielded positive results in all tests with pure cultures of diphtheria bacilli. It is reasonable to suppose that the amount of antitoxin in the control suspensions is responsible for the fact that more than twice as many test as control rabbits failed to react to the intracutaneous injection of *C. diphtheriae*. The technique described by Fraser and his co-workers decreases the reliability of the intracutaneous virulence test on rabbits.

The guinea pigs inoculated in these experiments were primarily intended as a control group. As variation in the reactions on test guinea pigs was noted, the number of animals was increased beyond the original plan. The results of these experiments are suggestive, but many more tests must be made in order to discover how frequently individual guinea pigs vary in their response to the inoculation of suspensions of the diphtheria bacillus. The public health laboratory technician is frequently called upon to test a few cultures for virulence. It would be economy in time and animals if one guinea pig could be used for these tests, as would be possible if both test and control suspension were inoculated at the same time. Since it seems evident that guinea pigs exhibit less variation, it may be possible to discover a safe adaptation of the method by reducing the amount of antitoxin in the control inoculum and the number of tests performed on one animal. This problem is being studied at this time and will be the subject of a later report.

CONCLUSIONS

1. The rabbit is not a suitable animal for the assay of virulence of *C. diphtheriae* by intracutaneous inoculation.

2. The use of a single rabbit for virulence testing by the simultaneous inoculation of test culture suspensions and control suspensions containing antitoxin does not yield reliable results.

3. Experimental evidence is presented which suggests that guinea pigs are less subject to variation in reaction than are rabbits. This will be investigated further.

4. The simultaneous intracutaneous inoculation of test culture suspensions and control suspensions containing antitoxin causes considerable variation in the reaction of guinea pigs but less than that noted in rabbits. This evidence corroborates that presented by the experiments with control guinea pigs which indicates that individual guinea pig variation may occur.

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RAPID METHOD FOR THE ISOLATION OF KERASIN FROM A GAUCHER SPLEEN*

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KERASIN is a characteristic component of the lipid fraction in Gaucher's disease and is used as a chemical confirmation of the diagnosis. For this reason, it is desirable to develop simple methods for its isolation and identification.

Kerasin is usually isolated from organs after preliminary drying and extraction of lipids. Rosenheim[1] accomplished both processes in one step by repeated extractions of wet organs with acetone. The kerasin was then isolated by extracting the dried residue with warm pyridine. More recently, McConnell, Forbes, and Apperly[2] extracted a dried spleen from a case of Gaucher's disease with acetone and ether repeatedly until the residue was lipid-free, and then extracted the kerasin from the residue with hot alcohol.

I employed both methods for the extraction of kerasin from a Gaucher spleen. The latter method gave a purer product. At the same time a method was devised for the extraction of kerasin which would entail no previous drying or repeated extractions with acetone and ether. It was found that kerasin could be removed completely and in a very pure state simply by drying the spleen with plaster of Paris and extracting the dried organ with hot 95 per cent alcohol. The alcohol on cooling deposited a bulky white precipitate of kerasin.

PROCEDURE

Plaster of Paris was added slowly and mixed with about 500 Gm. of finely ground spleen until a thick paste was formed. This was allowed to set for about an hour, then ground to a fine powder in a mortar. The powder was then extracted with 1,500 ml. of boiling 95 per cent ethyl alcohol. The suspension was filtered while hot through a large Buchner funnel, and the filtrate was cooled to 0° C. in a refrigerator. The precipitate formed on cooling was filtered with the aid of suction, and the alcoholic filtrate was used for further extractions of the splenic material. This process was repeated seven more times until no precipitate was formed on chilling the alcohol.

The precipitates of almost pure kerasin were combined and freed of lipid impurities by extraction over a period of four hours with ether in a Soxhlet extraction. The melting point of the dried material was found to be about 201° C. Repeated recrystallizations from methyl and ethyl alcohols gave a material which started to melt at 189° C., and melted with decomposi-

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tion at 204° C. Further purification, following the method of Bloom and Kern,[3] gave a product with the same melting point. A positive selenite test was obtained according to the procedure of Rosenheim.[1]

Hydrolysis with sulfuric acid in methyl alcohol gave a precipitate which, upon recrystallization from acetone, melted at 57° C. This corresponds closely with the melting point of the methyl ester of lignoceric acid which Rosenheim prepared by the same procedure and which was found to melt at 57° to 58° C.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RENAL DISEASE, Urea Reabsorption and Relation Between Creatinine and Urea Clearance in, Arkin, A., and Popper, H. Arch. Int. Med. 65: 627, 1940.

The clearance of urea and that of endogenous creatinine were compared in 87 cases of normal and of pathologic conditions in order to estimate the reabsorption of urea.

Urea reabsorption is constant during the same day, but not on different days. It is slightly diminished during fasting in moderate glomerular disorders, such as acute glomerulonephritis, benign hypertension, nephrosis, and extrarenal glomerular insufficiency. It is markedly diminished in many cases of severe damage to the kidneys, as in chronic nephritis, malignant hypertension, pyelonephritic contracted kidney, and extraglomerular acute nephritis (1 case). The lowest rate of reabsorption was found in pyelonephritic contracted kidney.

The decrease in urea reabsorption, usually combined with disturbed filtration and reabsorption of water, may depend on two factors: the decrease in the urinary concentration and the presence of damage to the kidneys.

With severest damage to the kidneys the urea clearance may exceed the creatinine clearance. This may be interpreted as an indication of urea secretion.

Uremia due to reabsorption in renal disease does not exist. On the contrary, stronger antiosmotic forces are effective at the tubular barrier against the back flow of urea. This mechanism is probably compensatory in order to prevent urea retention with decreased filtration.

ANGIITIS, Experimental Infectious, Winternitz, M. C., and LeCompte, P. M. Am. J. Path. 16: 1, 1940.

Various bacteria were introduced under aseptic technique into the adventitial tissue (more rarely on a thread into the lumen or into a doubly ligated stretch) of femoral, jugular, and carotid vessels of goats. By varying the virulence of the organism and the duration of the experiment, lesions of both artery and vein were obtained, ranging from acute suppurative and proliferative reactions with thrombosis to old, fibrous intimal plaques. A potential vascular pathway for passage of infection from vein to artery has been demonstrated in both goat and man by injection methods.

RHEUMATIC FEVER, Weltmann Reaction and Sedimentation Time During, of Childhood, Klein, R. I., Levinson, S. A., and Rosenblum, P. Am. J. Dis. Child. 59: 48, 1940.

The Weltmann reaction and the sedimentation rate were studied in 110 cases of rheumatic fever of childhood and in 10 cases of subacute bacterial endocarditis. The rheumatic fever group was made up of 26 cases of chorea, 32 cases of acute rheumatic arthritis without carditis, 39 cases of acute carditis, and 13 cases of cardiac decompensation.

1. The sedimentation rate with chorea, although usually normal, may at times be increased. This increased rate may be related to a previous infection. The Weltmann reaction with chorea is almost always either normal or increased.

2. Acute rheumatic arthritis and carditis are characterized by a rapid sedimentation rate and a low coagulation band. The coagulation band returns to normal before the sedimentation rate.

3. In cases of cardiac decompensation the sedimentation rate tends to slow, and the coagulation band tends to increase the values, depending on the severity of the decompensation in relation to the degree of infection.

4. The three pathologic stages of rheumatic fever, exudative, proliferative, and fibrotic, are reflected in the Weltmann reaction.

5. An increased sedimentation rate does not always mean that the rheumatic infection is still active. The patient may be convalescing in a phase which is pathologically termed the proliferative stage and still have a rapid sedimentation rate.

6. The Weltmann reaction is of value in the study of rheumatic fever, complementing the sedimentation rate; it may be of aid in the differentiation of subacute bacterial endocarditis from acute rheumatic carditis.

LEAD POISONING, Glycosuria in, Goettsch, E., and Mason, H. H. Am. J. Dis. Child. 59: 119, 1940.

A single case of lead poisoning with glycosuria is reported in detail. Studies carried out on this patient show that the height of the blood sugar was consistently normal or lower than normal, and that the urinary concentration of sugar remained relatively constant irrespective of the diet. The glycosuria persisted for five weeks, and the concentration of urinary sugar fell as convalescence progressed. The studies are compatible with the suggestion that the condition in lead poisoning may be classified as renal glycosuria. The suggestion is supported by a survey of 8 cases of lead poisoning with glycosuria in children. In 5 of the cases in which determinations were made, the blood sugar values were normal; in 3 cases in which dextrose tolerance curves were obtained, the curves were not of the diabetic type. Glycosuria does not seem to alter the prognosis in cases of lead poisoning in children.

COMPLEMENT FIXATION, A Simplified Technic, Its Sensitivity and Specificity, Gillman, R. L., Boerner, F., and Lukens, M. Arch. Dermat. & Syph. 41: 32, 1940.

The authors have described a new modification of the complement fixation test for syphilis, which has been determined to be more sensitive than the Kolmer modification and the Kahn flocculation test, although less so than the Eagle flocculation test. There has been no loss in specificity.

The Boerner-Lukens modification of the complement fixation test tends toward simplification through the use of an easily prepared antigen and the addition of the reagents in combination. There ensues less labor and cost and the addition of improved supervision.

In a series of 1,500 tests, there were 20 instances of lack of accord in specificity, all of which are analyzed against the clinical status of the patient.

The continuance of the use of two or three tests for the serologic evaluation of syphilis is urgent.

A closer rapport is necessary between the serologist and the clinician, in order that the patient's interests may best be served.

SULFAPYRIDINE, Pathologic and Histologic Changes Following Oral Administration of, Antopol, W., and Robinson, H. Arch. Path. 29: 67, 1940.

Urolithiasis occurs after the feeding of sulfapyridine to monkeys, rabbits, and rats. The concretions can be either redissolved or washed out. It cannot be ascertained whether the formation of the urolith is always an independent precipitation process, or whether it is at times dependent on primary degenerative or vascular changes in the kidney.

TUBERCULIN TEST, The Patch Test and Mantoux Intradermal Test, Pearse, A. J., Fried, R. L., and Glover, V. A. J. A. M. A. 114: 227, 1940.

Seven hundred and twelve school children were given both the tuberculin patch test and the Mantoux test, first and second strength purified protein derivative.

Six hundred and sixteen had either both tests positive or both negative, the percentage correlation between the two tests in this series being 87 plus.

Sixty-seven had positive patch and negative Mantoux tests.

The tuberculin patch test has a high degree of correlation with the Mantoux test and appears to give 7 per cent more positives than the Mantoux.

The authors conclude that the tuberculin patch test is as reliable as the Mantoux. Its ease of application and nontraumatizing character make it superior to the Mantoux test in other ways.

They believed for these reasons that the tuberculin patch test is the method of choice in large scale tuberculin testing, especially for children.

CEREBROVASCULAR ACCIDENTS, Duration of Life After, Newbill, H. P. J. A. M. A. **114:** 236, 1940.

Sudden death (within two hours) from cerebrovascular accidents is the exception rather than the rule.

Hemorrhage is more likely to be responsible if death occurs within twenty-four hours of the onset of symptoms, while thrombosis is far more common in patients surviving for more than one month.

The average survival period after thrombosis is approximately fifteen times as long as after hemorrhage or embolism.

There was a distinct difference in the survival periods of the white (105.5 days) and the Negro races (64.8 days). This difference was confined mainly to the patients with thrombosis.

There was an equally distinct difference between males (57.3 days) and females (129.1 days). Here again the essential difference was found to be in the group with thrombosis.

When apoplexy occurred between the ages of 21 and 60, the average survival period was one month or longer. Death usually occurred within one month when the patient was below 21 or above 60 years old. The maximal survival period fell within the 30 to 40 year year group, here averaging 141.0 days.

As would be expected, the location of the lesion and the number of lesions were important determining factors in the survival period.

PREGNANCY, Study of "False" Friedman Tests for, Randall, L. M., Magath, T. B., and Pansch, F. N. J. A. M. A. **114:** 471, 1940.

The Friedman test is based primarily on the fact that if anterior pituitary-like hormones are present in the urine in certain amounts, they will be demonstrable by typical reactions in the ovary of the rabbit. While the test is not a test of pregnancy from an academic point of view, to all practical purposes it is so if the correct technique is followed.

The Friedman test is, therefore, based on quantitative considerations. Excretion of the gonadotropic principle of the anterior lobe of the hypophysis in the urine in excess amounts may be responsible for a positive Friedman reaction in the absence of pregnancy.

Among the several physiologic and pathologic states other than normal pregnancy which may give a positive reaction are hydatidiform mole, chorio-epithelioma, the menopause, menstrual disorders, such as primary ovarian failure, treatment with preparations of the anterior lobe of the pituitary gland, and errors in technique.

A negative reaction to the Friedman test prior to the seventh week after the last menstrual period may not be conclusive, although authentic positive reactions may be obtained much earlier, frequently within four weeks after impregnation. A test that gives negative results before the seventh week should be repeated later.

PNEUMOCOCCIC INFECTIONS, Treatment of, in Children With Sulfapyridine, Christian, H. S., Jorgensen, G. M., and Ellis, C. Am. J. Dis. Child. **59:** 1, 1940.

Sulfapyridine was used in the treatment of 140 patients with pneumococcic infections. No patients were left untreated for controls. Among the 100 patients with pneumonia only one death occurred. For 9 of the 13 patients with pneumococcic bacteriemia the blood

cultures were negative within twenty-four hours after treatment was started. Some of the limitations of sulfapyridine were demonstrated in 2 cases of pneumococcic meningitis in which the patients died, and in a case of pneumonia in which bacteriemia persisted during five days of treatment before antipneumococcus serum was given. Studies on absorption and excretion of sulfapyridine are presented.

BLOOD, Human Serum as a Substitute for, in the Treatment of Hemorrhage and Shock, Levinson, S. O., Neuwelt, F., and Necheles, H. J. A. M. A. **114**: 456, 1940.

Immediate restoration of fluid volume following extensive hemorrhage prevents the development of secondary shock.

Delay in restoration of fluid volume following extensive hemorrhage permits the development of secondary shock.

Crystalloid solutions are ineffective when secondary shock develops after severe hemorrhage.

Serum is an effective agent in combating all the effects of severe hemorrhage and resultant secondary shock except the loss of red blood cells.

Erythrocyte loss is not serious unless very extensive.

Whole blood, the best restorative agent, requires time-consuming laboratory tests preliminary to administration, leading to an unavoidable delay counteracting its ultimate beneficial effects.

Serum may be given in massive amounts without any delay or preliminary testing.

Serum can be used as an interval measure preceding blood transfusions, or as a substitute measure when blood is not available or when erythrocyte loss is not too extensive.

Human serum, as a blood substitute, should find wide use not only in civil emergencies but particularly in time of war at the battle front.

NEPHROSIS, Concentrated Human Blood Serum as a Diuretic in, Aldrich, C. A., and Boyle, H. H. J. A. M. A. **114**: 1062, 1940.

Seven additional patients with pure nephrosis were treated with four times concentrated pooled human serum.

Diuresis usually occurred in these patients and went on to complete elimination of edema fluid as a rule, sometimes to complete recovery from the nephrosis.

Patients with other types of renal edema, notably those with any hematuria, were not benefited.

When the cases first reported in the present paper were listed, together with nine cases reported in 1938, according to the elapsed time between first symptom and first treatment with concentrated serum, it became evident that those treated earliest had a much more favorable subsequent course. This suggests that this method should be used by first choice rather than as a last resort.

Reactions were insignificant unless hemolysis had occurred in the serum. This is shown by its being definitely red.

If the patient showed evidence of acute infection, the serum was ineffective.

SULFANILAMIDE, The Mode of Action of, Green, H. N. Brit. J. Exper. Path. **21**: 38, 1940.

It is suggested on the basis of numerous experiments, mainly with *Br. abortus*, that whether or not a bacterial culture survives in the presence of sulfanilamide depends on three factors:

1. The rate of proliferation in the initial growth phase. If the bacterial density in a fluid medium reaches a certain level (''critical'' bacterial density), an amount of some antisulfanilamide factor, sufficient to prevent any further bactericidal action by the sulfanilamide present, accumulates in the medium. The amount of this factor progressively increases, and at a certain concentration bacterial reproduction is resumed.

2. The rate at which sulfanilamide reaches a bacteriostatic concentration in the cell. This depends mainly on the amount of sulfanilamide in the medium, and possibly to some extent on the nature of the bacterial envelope.

3. The permeability of the cell membrane to the antisulfanilamide factor. This factor is released and possibly formed during autolysis of the dead or dying cell, and diffuses out into the surrounding medium. The rate at which it is released may be determined by the chemical nature of the capsule and/or cell membrane peculiar to the bacterial species or strain.

Bacterial extracts containing this antisulfanilamide factor powerfully stimulate the reproductive rate of many bacteria, and the factor is, therefore, termed provisionally "P" factor (proliferation or pullulation factor).

The "P" factor appears to be identical with the antisulfanilamide factor, and it is suggested that it stimulates the same enzyme reaction in the bacterial cell which sulfanilamide inhibits. This reaction is probably a fundamental one in the metabolism of most or all bacteria, but not in the metabolism of other living cells

The "P" factor is not species specific in either its source or its action.

It is very probable that the sensitivity to sulfanilamide of any bacterial species or strain is conditioned by the rate at which the autolyzing organism liberates "P" factor.

Under certain conditions a balance may exist between "P" factor and sulfanilamide, and the organism is then incapable of reproduction. The living nature of the organism is only revealed when the concentration of "P" factor increases.

A sulfanilamide-resistant strain of *Br. abortus* appears when the organism survives for some time in the presence of sulfanilamide. The resistance of the strain increases the longer it survives in sulfanilamide. Resistant strains do not develop in the presence of "P" factor alone.

SULFAPYRIDINE CALCULI, The Production of, in Man, Plummer, N., and McLellan, F. J. A. M. A. **114:** 943, 1940.

Two patients had renal calculi after sulfapyridine treatment, proved at post-mortem examination and by pyelography, respectively. Both patients had hematuria and one also had renal colic; one had had only 11 Gm. of the drug and the other 555 Gm.

Pyelograms in one of the cases showed calculi, nonopaque to x-rays. The serial study indicates that later the calculi dissolved or were washed out, and the kidney function returned to normal.

The features of these two cases, together with the experimental studies reported by other investigators, indicate that hematuria and other urinary symptoms occurring after sulfapyridine administration are caused by the deposition of sulfapyridine crystals and concretions in the kidneys and ureters.

Frequent examinations of the urine, particularly for red blood cells, should be made during sulfapyridine therapy. The drug should be withheld or given very cautiously if hematuria occurs, or if the patient is known to have a diminished kidney function.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Diseases of the Skin*

A BOOK which is still widely used twenty-three years after its first appearance, and which has gone through a total of ten editions, requires no introduction. The tendency of such volumes is to become progressively larger in succeeding editions. This book has followed this tendency, containing now over 1,500 pages. However, this does not imply simple additions to previous pages, for the present edition has been revised and reset. Its large size is merely evidence of the tremendous increase of knowledge and observations in the field.

The illustrations are startlingly good and most abundant. This is an essential feature of all good texts on dermatology.

New aspects in dermatology that have been adequately covered include the allergic dermatoses and dermatoses associated with constitutional maladies.

This is an excellent reference volume.

Mathews' Physiological Chemistry†

TWENTY-FIVE years ago "Mathews" was a standard text in physiological chemistry, used by the present reviewer in his student days. With the continuous avalanche of new discoveries and the more or less continuous changing of teachers, many of whom write and prescribe their own texts, a quarter century is a long life for any book in medicine. It must very nearly approach a record. The first edition appeared in 1915. From then until 1932 either a reprinting or a new edition appeared every year except 1929. Then came a lapse of several years, until the present sixth edition appeared in September, 1939.

One can follow in his imagination the mental attitude of an author who, struggling year after year in his successive revisions, at last becomes surfeited with what had started as a pastime and had ended as a task. After the lapse of sufficient time he again becomes interested and enthusiastic. The fresh approach enables him to start at the beginning, with no subconscious regrets at cutting widely in many places. The result is a textbook of biochemistry, up to date, which stands as a monument to a pioneer and leader in the field.

There are two general parts, with many subheadings. The first is "The Chemistry of Protoplasm and the Cell"; the second is "The Mammalian Body Considered as a Mechanism."

The section on Laboratory Outlines and Laboratory Methods has been omitted and is published separately by F. C. Koch. Even with this omission the volume contains nearly 1,500 pages.

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*Diseases of the Skin. By Richard L. Sutton, M.D., Sc.D., LL.D., F.R.S. (Edin.), Professor of Dermatology, University of Kansas, School of Medicine; and Richard L. Sutton, Jr., A.M., M.D., L.R.C.P. (Edin.), Associate in Dermatology, University of Kansas, School of Medicine. Cloth, ed. 10, 1,549 pages, with 1,452 text illustrations and 21 color plates, revised, enlarged, and reset. The C. V. Mosby Company, St. Louis, Mo., 1939.

†Physiological Chemistry: A Text-book for Students. By Albert P. Mathews. Cloth, ed. 6, 1,488 pages, with 113 illustrations, $8.00. William Wood & Co., Baltimore, Md., 1939.